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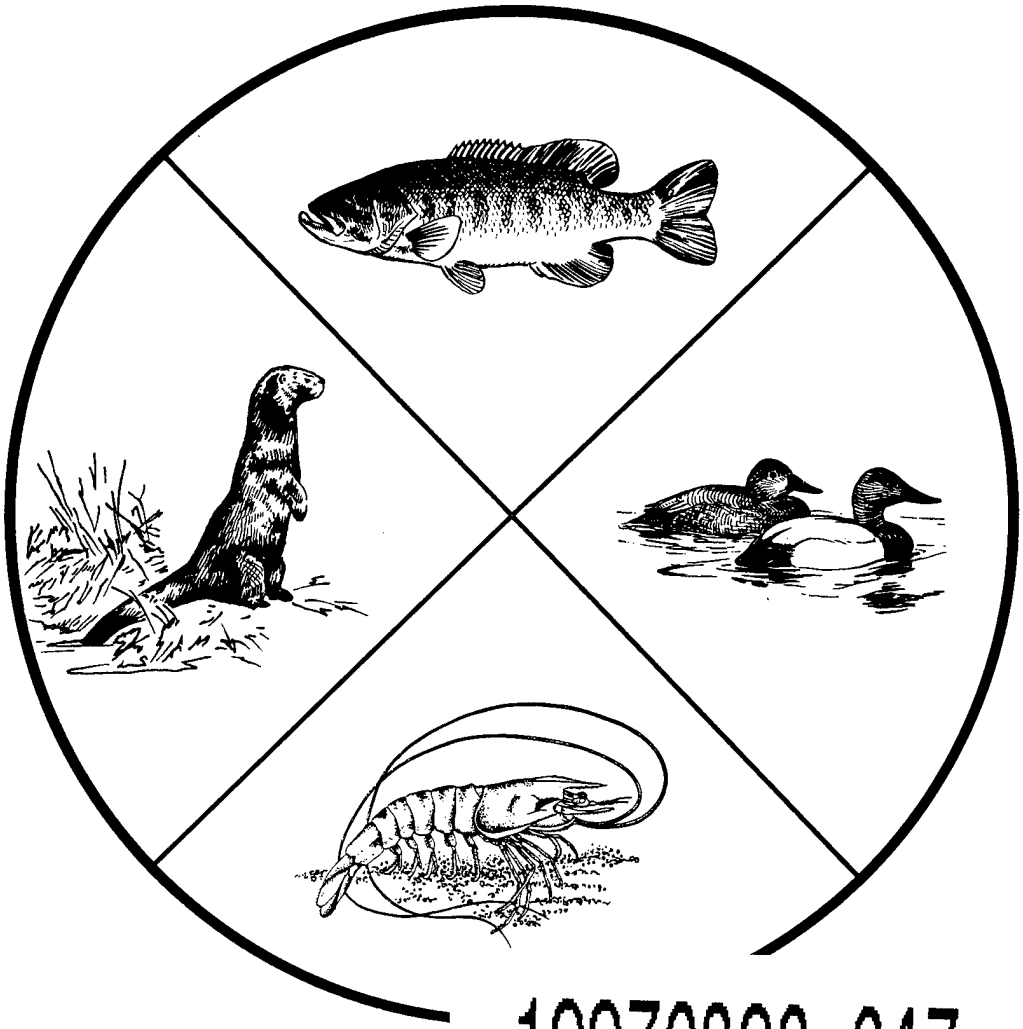
Contaminant Hazard Reviews
Report 25

Diflubenzuron Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review



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By

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Diflubenzuron Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review

by

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Abstract. Diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea), also known as dimilin, is a potent broad-spectrum insect growth regulator that interferes with chitin synthesis at time of molting and is effective in controlling immature stages of insects. Diflubenzuron was approved for domestic use in 1976 to control gypsy moth (*Lymantria dispar*), and in 1979 against the cotton boll weevil (*Anthonomus grandis*). By 1989 this compound was also registered for domestic use against mosquitos, forest Lepidoptera, mushroom flies, and leaf-eating insect pests of citrus, woody ornamentals, vegetables, and fruit.

Diflubenzuron seldom persists for more than a few days in soil and water. When used properly in forest management, it is unlikely to be leached into ground water from the application site. Degradation in water and soil is most rapid when small particle formulations are applied; microorganisms are abundant; and at elevated pH, temperature, and organic loading. Chemical and biological processes initially yield 2,6-difluorobenzoic acid and 4-chlorophenylurea. Soil degradation processes and plant and animal metabolism involve further conversion of these compounds to 2,6-difluorobenzamide and 4-chloroanaline. Ultimately, the end products are either conjugated into mostly water soluble products or biologically methylated.

Diflubenzuron applied to foliage of terrestrial plants tends to remain adsorbed for several weeks with little or no absorption or translocation from plant surfaces; loss occurs mainly from wind abrasion, rain washing, or shedding of senescent leaves. Among terrestrial insects, there is great variability in sensitivity to diflubenzuron. Sensitive pestiferous species of insects die at topical applications of 0.003–0.034 μg per larvae or after consuming diets containing 0.1 mg/kg. Some beneficial insects, such as the honey bee (*Apis mellifera*), are adversely affected at 1 mg/kg fresh weight (FW) of diet.

Diflubenzuron application rates between 28 and 56 g/ha (0.025–0.05 pounds per acre) or 2.5 to 16 μg/L are highly effective against pestiferous aquatic dipterans, including representative chaoborids, chironomids, and culicids. These same dosages temporarily suppress nontarget populations of cladocerans, copepods, mayfly nymphs, corixids, and springtails; population recovery is usually complete within 80 days. In general, crustaceans were the most sensitive nontarget aquatic organisms tested. Adverse effects on crustacean growth, survival, reproduction, and behavior occur between 0.062 and 2 μg/L. Next in sensitivity are mayflies, chironomids, caddisflies, and midges; concentrations between 0.1 and 1.9 μg/L produce low emergence and survival. Moderately resistant to diflubenzuron are larvae of diving beetles, dragonfly adults and naiads, ostracods, spiders, backswimmers, and water boatmen. Relatively tolerant of diflubenzuron (i.e., no observable adverse effects at $\leq$45 μg/L) are the algae, molluscs, fishes, and amphibians. High accumulations occur on some aquatic plants during exposure to 100 μg/L and in fish during exposure to 1 to 13 μg/L, but all species in these groups seem unaffected by elevated body burdens and grow and metabolize normally.

Birds seem comparatively resistant to diflubenzuron: acute oral LD50 doses exceed 2,000 mg/kg body weight (BW); dietary concentrations <4,640 mg/kg FW are tolerated for at least 8 days; and forest birds seem unharmed by recommended diflubenzuron application procedures to control pestiferous insects. Intraspecies differences in ability to metabolize diflubenzuron are probably large; different strains of domestic chickens show significant differences in ability to accumulate and retain this compound.

No data were found on diflubenzuron effects on mammalian wildlife. However, studies on

small laboratory animals and domestic livestock indicate no observable effects in cows (*Bos bovis*) given 0.25 mg/kg BW daily for 4 months, in rabbits (*Oryctolagus cuniculus*) given 4 mg/kg BW daily on days 6 to 18 of gestation, in dogs (*Canis familiaris*) fed diets containing 40 mg/kg for 13 weeks (equivalent to 1.6 mg/kg BW daily), in rats (*Rattus* spp.) fed diets containing 160 mg/kg for 2 years, and in rabbits and rodents given single oral or dermal doses <2,000 mg/kg BW. All experimental studies conducted with laboratory animals indicate that diflubenzuron is nonmutagenic, nonteratogenic, and noncarcinogenic. Adverse effects occur in dogs fed diets containing 160 mg/kg (6.2 mg/kg BW daily) for 13 weeks (abnormal blood chemistry), in mice (*Mus* spp.) given 125 mg/kg BW daily for 30 days (hepatocellular changes), in rabbits fed diets of 640 mg/kg for 3 weeks (abnormal hemoglobin), and in rats given 5,000 mg/kg BW daily for 13 weeks (abnormal hemoglobin). Elevated tissue residues—but no other measurable effects—occur in cows given 0.05 to 0.5 mg/kg ration for 28 days or 1 to 16 mg/kg BW for 4 months, in pigs (*Sus* spp.) given a single oral dose of 5 mg/kg BW, and in sheep (*Ovis aries*) given a single oral dose of 10 mg/kg BW.

Criteria now recommended for protection of various species include the following: dietary loadings, in mg/kg FW ration, of <0.05 for human health, <0.05 for livestock, <1 for honey bees, and <5 for poultry; seawater concentrations <0.1 μg/L for estuarine crustacean larvae; and, for all aquatic life, restricted or prohibited use of diflubenzuron in salt-marsh mosquito breeding areas and on agricultural lands less than 5 km from coastal areas. No criteria are available or proposed for protection of avian and mammalian wildlife against diflubenzuron, probably because of an incomplete toxicological data base.

Key words: Diflubenzuron, dimilin, benzoylphenyl urea, insecticide, ovicide, wildlife, aquatic organisms, ecotoxicology, criteria.

Compounds collectively known as insect growth regulators have been recognized, in recent years, as important new insecticides. These compounds include juvenile hormone mimics, antijuvenile hormone analogs, and chitin synthesis inhibitors. The most widely studied chitin synthesis inhibitor, and the only one currently registered for use against selected insect pests in the United States, is diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea), also known as dimilin (Christiansen 1986; Touart and Rao 1987). Chitin is a major component of the tough outer covering, or cuticle, of insects. As insects develop from immature larvae to adults, they undergo several molts, during which new cuticles are formed and old ones shed.

Diflubenzuron prevents successful development by inhibiting chitin synthetase, the final enzyme in the pathway by which chitin is synthesized from glucose (Marx 1977; Ivie 1978).

Diflubenzuron is highly effective against larval stages of many species of nuisance insects. It is used extensively to control mosquitos, midges, gnats, weevils (including the cotton boll weevil, *Anthonomus grandis*), various beetles, caterpillars of moths and butterflies (especially the gypsy moth, *Lymantria dispar*), flies, and rust mites (Marx 1977; Ivie 1978; Veech 1978; Schaefer et al. 1980; Opdycke et al. 1982a; Muzzarelli 1986). In Maryland, for example, more than 30,000 ha are sprayed annually to control gypsy moths (Swift et al. 1988a). In general, less than 140 g/ha (2 ounces per acre) of diflubenzuron is sufficient to control susceptible species, although affected larvae do not die until they molt (Marx 1977).

Most authorities agree that diflubenzuron has low mammalian toxicity, is not highly concentrated through vertebrate food chains or by absorption from water, remains stable on foliage, and seldom persists for extended periods in soil and water (Marx 1977; Ivie 1978; Schaefer et al. 1980). Chitin synthesis inhibitors, however, are not specific to insect pests. Beneficial insects also produce chitin, as do all arthropods, including spiders, crabs, crayfish, lobsters, shrimp, daphnids, mayflies, stoneflies, barnacles, copepods, and horseshoe crabs. All of these groups are adversely affected by diflubenzuron, including effects on survival, reproduction, development, limb regeneration, and population growth (Farlow 1976; Marx 1977; Christiansen 1986; Cunningham 1986; Muzzarelli 1986; Touart and Rao 1987; Weis et al. 1987).

This report was prepared in response to requests for information on diflubenzuron from environmental contaminant specialists of the U.S. Fish and Wildlife Service. It is part of a continuing series of brief reviews on chemicals in the environment, with emphasis on fishery and wildlife resources.

Environmental Chemistry

General

Diflubenzuron breakdown by hydrolysis, soil degradation, or plant and animal metabolism initially yields 2,6-difluorobenzoic acid and 4-chlorophenylurea. Ultimately, the end products are either conjugated into mostly

water soluble products or are biologically acylated and methylated. At extremely low doses, diflubenzuron selectively inhibits the ability of arthropods to synthesize chitin at the time of molting, producing death of the organism from rupture of the cuticle or starvation. Other organisms that contain chitin (i.e., some species of fungi and marine diatoms), or polysaccharides similar to chitin (i.e., birds and mammals), seem unaffected.

Mobility and leachability of diflubenzuron in soils is low, and residues are usually not detectable after 7 days. Degradation is most rapid when small-particle (2–5 μm) formulations are applied and soil bacteria are abundant. In water, diflubenzuron usually persists for only a few days; degradation is most rapid under conditions of high organic and sediment loadings, and elevated water pH and temperature.

Chemical and Biochemical Properties

Selected chemical properties of diflubenzuron are listed in Table 1.

Diflubenzuron degradative pathways are almost entirely through cleavage between the carbonyl and amide groups of the urea bridge. Ultimately, the end products are either conjugated into predominantly water soluble products or are acylated and methylated biologically (Metcalf et al. 1975). Hydrolysis, soil degradation, and plant and animal metabolism of diflubenzuron yield the same initial products: 2,6-difluorobenzoic acid and 4-chlorophenylurea. Soil degradation and plant and animal metabolism involve further conversion of these compounds to 2,6-difluorobenzamide and 4-chloroaniline (Schaefer et al. 1980; Gartrell 1981; Figure).

Interspecies variations in ability to metabolize diflubenzuron are common, as judged by metabolic patterns in rat, cow, and sheep. In all three species hydroxylation of either aromatic ring and scission of the ureido bridge constituted the main metabolic pathways. In cow and rat the prevailing route was ring hydroxylation; in sheep it was the scission reaction. In cow and sheep about half the 2,6-difluorobenzoyl moiety excreted in urine was conjugated to glycine, but in rat the acid was excreted largely unchanged. In sheep, where cleavage splitting of the diflubenzuron molecule was the primary metabolic route, there was no evidence of 4-chlorophenylurea or 4-chloroaniline in urine (Willems et al. 1980). More infor-

Table 1. *Chemical and other properties of diflubenzuron.*[a]

Variable	Data
Chemical names	1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea; N-[[(4-chlorophenyl)amino] carbonyl]-2,6-difluorobenzamide); 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl) urea
Alternate names	Deflubenzon, Diflubenuron, Dimilin, DU 112307, Duphar BV, ENT-29054, Largon, Micromite, OMS 1804, PDD 6040-I, PH 60-40, TH 6040
Action	Insecticide, larvicide, ovicide; insect growth regulator acting by interference with deposition of insect chitin
CAS number	35367-38-5
Empirical formula	$C_{14}H_9ClF_2N_2O_2$
Molecular weight	310.68
Formulations	Granular, oil-dispersable concentrate; wettable powder
Manufacturing process and impurities	Produced by reaction of 2,6-difluorobenzamide with 4-chlorophenyl isocyanate. The technical product is 95% pure. Impurities are of low toxicological concern in terminal residues
Stability	Stable under sunlight and in neutral or mildly acidic solutions; unstable in strong basic solutions.
Physical state	White crystalline solid
Melting point	210–230°C (technical); 230–232°C (pure)
Solubility	
Water	0.1–0.2 mg/L at 20°C; 1.0 mg/L at 25°C
Polar organic solvents	Moderate to good
Octanol/water partition coefficient	3,500

[a] Metcalf et al. 1975; Farlow 1976; Johnson and Finley 1980; Gartrell 1981; Hudson et al. 1984; Mayer 1987; Poplyk 1989.

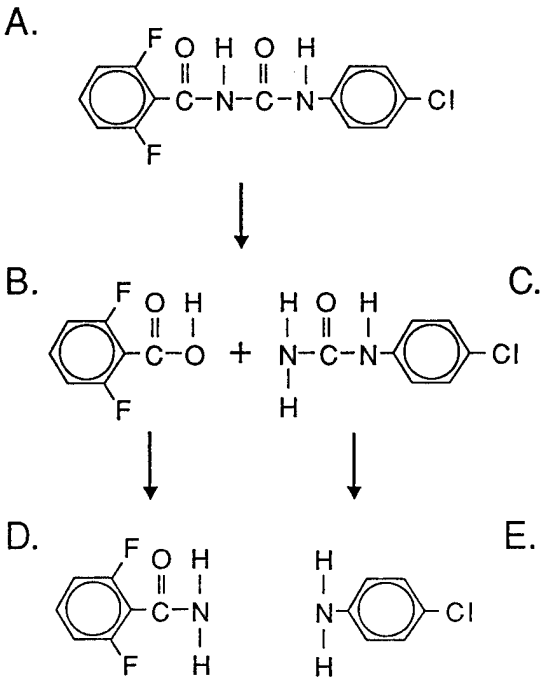

Figure. Generalized degradation pattern for diflubenzuron. Diflubenzuron (*A*) degrades initially to 2,6-difluorobenzoic acid (*B*) and 4-chlorophenylurea (*C*). 2,6-difluorobenzoic acid degrades to 2,6-difluorbenzamide (*D*); 4-chlorophenylurea degrades to 4-chloroaniline (*E*).

mation on degradation and metabolic pathways of diflubenzuron is given in Metcalf et al. (1975), Schooley and Quistad (1979), Ivie et al. (1980), Willems et al. (1980), Franklin and Knowles (1981), and Jenkins et al. (1986).

The benzoylphenylureas—including diflubenzuron—control target insect populations at extremely low doses by selectively inhibiting their ability to synthesize chitin-bearing parts. Ingested diflubenzuron has no apparent adverse effects until the molting process is under way.

Diflubenzuron caused increases in cuticle chitinase and cuticle phenoloxidase activity, producing a softened endocuticle through reduction of its chitin content and a hardened exocuticle as a result of increased phenoloxidase activity (Farlow 1976). Diflubenzuron inhibits serine protease, thus blocking the conversion of chitin synthetase zymogen into an active enzyme (Cunningham 1986; Muzzarelli 1986). Insect larvae treated with diflubenzuron develop cuticles that are unable to withstand the increased turgor occurring during ecdysis and that fail to provide sufficient muscular support during molting. These larvae are unable to cast their exuviae, resulting in death from starvation or rupture of the new, delicate, malformed cuticle (Farlow 1976). In addition to terrestrial insects, diflubenzuron is toxic to a wide variety of aquatic insects and crustaceans (Swift et al. 1988a, 1988b), but it doesn't seem to affect other organisms that contain chitin, including fungi (Muzzarelli 1986) and marine diatoms (Montgomery et al. 1990).

Chitin is a polymer of N-acetylglucosamine (AGA), and it rivals cellulose as the most abundant biopolymer in nature. Measured chitin concentrations in marine waters range between 4 and 21 μg/L, and planktonic crustaceans are the most significant source of chitin in the sea (Montgomery et al. 1990). Insect chitin is synthesized during phosphorylation by uridine disphospho N-acetyl glucosamine (UDPAGA)—the immediate precursor of chitin (Crookshank et al. 1978). Diflubenzuron inhibits the incorporation of chitin precursors into chitin, with a resultant accumulation of UDPAGA (Crookshank et al. 1978). Chitin is not found in vertebrates, although several important polysaccharides similar to chitin are found, including hyaluronic acid (HA). Hyaluronic acid is found in skin, synovial fluid, connective tissue, vitreous humor, and the covering of the ovum. Hyaluronic acid is a polysaccharide compound of alternating groups of glucuronic acid and AGA; the immediate precursor for glucuronic acid is uridine diphospho-glucuronic acid and that for AGA is UDPAGA. Because UDPAGA is used in the synthesis of chitin by insects and of HA by vertebrates, and because diflubenzuron interferes with the incorporation of UDPAGA into chitin by insects, diflubenzuron may interfere with the formation of HA in birds (Crookshank et al. 1978).

Persistence in Soil and Water

Mobility and leachability of diflubenzuron in soils is low, and residues are usually not detectable after 7 days. In water, half-time persistence (Tb 1/2) is usually less than 8 days and lowest at elevated temperatures, alkaline pH, and high sediment loadings (Table 2). Increased concentrations of diflubenzuron in soils and waters are associated with increased application frequency, flooding of treated supratidal areas, wind drift, and excessive rainfall (Cunningham 1986).

Diflubenzuron is persistent in postharvest soils during winter and spring months, especially if associated with plant litter; concentrations decline rapidly with the onset of high summer temperatures to <0.3 mg/kg DW soil in summer (Bull and Ivie 1978; Bull 1980).

Diflubenzuron particle size and soil flora may be important in the soil degradation process. Diflubenzuron adsorbed to smaller particles of 2 μm diameter had a short Tb 1/2 of 3–7 days; diflubenzuron adsorbed to larger particles (10 μm diameter) persisted for 8–16 weeks. Diflubenzuron adsorbed to particles of 2 μm diameter had a low rate of degradation in sterile soils (<6% in 4 weeks), but in nonsterile soils 98% degraded in the same period, suggesting that soil bacteria are important in the degradation process (Cunningham 1986). In Canada, data on mobility of a pesticidal chemical in forest soil must be collected before it can be registered for use under the

Table 2. *Diflubenzuron persistence in soil and water.*

Sample, initial concentration, and other variables	Persistence	Reference[a]
Soil		
0.08 g/ha, aerial spray, single application	Values always <0.05 mg/kg up to 14 days after spraying	1
22.4 g/ha (0.02 pounds per acre) applied four times, one month between treatments	Residues, in mg/kg, after first treatment were 0.2 at 1 day and 0.016 at 7 days. Residues at time of fourth treatment were nondetectable (ND) at start, 0.01 at 1 h, 0.02 at 1 day, and ND between days 3 and 56	2
44.9 g/ha (0.04 pounds per acre) applied four times, 2 weeks between treatments	Maximum residues, in mg/kg, after first treatment were 0.07 at 1 day and 0.05 at 7 days. After fourth treatment, residues were 0.04 at start, 0.09 at 3 days, and ND between days 7 and 56	2
70, 210, or 630 g/ha applied once to sandy loam forest soil or clay loam forest soil, plus water equivalent to 50.8 cm of precipitation	Mobility of diflubenzuron was low and did not increase with dosage. No residues detected below 10 cm or in leachates in either soil type at all dosage levels. At 70 g/ha, all residues were found in the top 2.5 cm; at 630 g/ha, 4–9% moved below 2.5 cm in sandy loam (mobility was lower in clay loam)	3
Distilled water		
100 μg/L, 37°C	No degradation at pH 4 in 8 weeks; Tb 1/2 was about 7 days at pH 6 and <3 days at pH 10. Major degradation products were 4-chlorophenylurea and 2,6-difluorobenzoic acid; small amounts of 2,6-difluorobenzamide and a quinazolinedione product were also formed	4
Pasture water		
22–45 g/ha (0.02–0.04 pounds per acre), single application	Maximum concentrations, in μg/L, were 8.8 in 1 h, 7.1 in 24 h, 3.9 in 48 h, and 2.6 in 72 h; most treatments produced ND (<1 μg/L) residues in 24 h	5
45 g/ha (0.04 pounds per acre), single application	Concentrations, in μg/L, were 4 at start, 36 at 1 h, 9 at 24 h, and 6 at 14 days	1
45 g/ha, applied four times at 2-week intervals	Maximum concentrations, in μg/L, were 7.4 at 1 h after first treatment, 1.3 at 1 h after second application, 2.9 at 1 h after third treatment, and 6.4 at 1 h and 0.9 at 1 day after last treatment	1
80 g/ha, single application	Concentrations, in μg/L, of diflubenzuron declined from 20.3 at 1 h to 2.4 at 4 days; 4-chlorophenylurea increased slightly from 5.6 to 7.2 during this interval; 4-chloroaniline increased from 0.7 at 1 h to 2.6 at 4 days	6
Pond water		
2.5 μg/L	Concentration after treatment was 1.9 μg/L; after 2 weeks it was 0.5 μg/L	7
5 μg/L	Concentration immediately after treatment was 4.6 μg/L; after 2 weeks it was 0.3 μg/L	7
10 μg/L	Initial concentration in medium declined from 9.8 μg/L to 0.2 μg/L after 2 weeks	7

Table 2. *Continued.*

Sample, initial concentration, and other variables	Persistence	Reference[a]
Seawater		
10 μg/L, sediments present	Tb 1/2 of 5.3 days; <0.7 μg/L in 19 days; <0.5 μg/L in 22 days	8
10 μg/L, sediments absent	Tb 1/2 of 17.8 days	8
100 μg/L	Tb 1/2 of 7.9 days at 38°C, and 35 days at 24°C	9
45 g/ha	Tb 1/2 of 10 days	9

[a] 1, Booth and Ferrell 1977; 2, Schaefer and Dupras 1977; 3, Sundaram and Nott 1989; 4, Ivie et al. 1980; 5, Schaefer and Dupras 1976; 6, Schaefer et al. 1980; 7, Apperson et al. 1978; 8, Cunningham et al. 1987; 9, Cunningham and Myers 1986.

Canadian Pest Control Products Act in order to assess its potential for groundwater contamination (Sundaram and Nott 1989). Diflubenzuron used properly in forest management is unlikely to be leached into ground water from a site of application (Sundaram and Nott 1989).

Water concentrations of diflubenzuron in treated ponds are significantly higher in surface and middle samples than in bottom samples during the first 5 h after treatment; however, after 24 h, distribution is about the same for all depths (Colwell and Schaefer 1980). Diflubenzuron persists for only a few days in pasture waters at 22–45 g/ha applied to control pasture mosquitos (*Aedes nigromaculis, A. melanimon*); hydrolysis and adsorption onto organic matter limit persistence in water (Schaefer and Dupras 1977). Water temperature and pH significantly affect persistence of diflubenzuron, though not always in a linear fashion. Degradation is most rapid at elevated temperatures and alkaline pH values. Half-time persistence of diflubenzuron at pH 7.7 and various thermal regimes is 8 days at 38° C, 35 days at 24°, and 29 days at 10°; at pH 10, Tb 1/2 values are 2 days at 38°, 14 days at 24°, and 32 days at 10°; degradation is negligible at pH 4, and at low temperatures regardless of pH (Cunningham 1986). In water, as in soil, small-particle (2–5 μm diameter) diflubenzuron formulations, such as WP-25%, degrade rapidly, usually in 2–8 days (Cunningham 1986). Larger-particle sand-granule formulations, developed for use in mosquito control programs wherein the compound needs to penetrate thick vegetation to reach the water, reduce drift during application, and also provide slower release of diflubenzuron into aquatic habitats (Cunningham 1986).

The presence of sediments in diflubenzuron marine microcosms results in rapid removal from seawater and ultimately a reduction in mortality of larval crustaceans (Table 2; Cunningham et al. 1987). But marine sediments that exceed 200 g diflubenzuron/kg—levels normally encountered at application rates for control of salt-marsh mosquitos—could be detrimental to juvenile and adult crustaceans that consume detritus and organic matter on the surface of the marsh or at the water-sediment interface (Cunningham and Myers 1986; Cunningham et al. 1987).

Uses

Diflubenzuron effectively inhibits molting in many species of insect pests, especially among the Lepidoptera, Coleoptera, and Diptera. In the United States diflubenzuron was approved for use by the U.S. Environmental Protection Agency (EPA) against the gypsy moth in 1976, the cotton boll weevil in 1979, and foliar feeders on soybeans in 1982. By 1989 diflubenzuron was also registered for domestic use against mosquitos, forest Lepidoptera, mushroom flies, and certain leaf-eating insect pests of citrus, woody ornamentals, vegetables, and fruits (Bull 1980; Nimmo et al. 1980; Gartrell 1981; Cunningham 1986; Muzzarelli 1986; Webb and Wildey 1986; Wilson and Costlow 1987; Martinat et al. 1988; Poplyk 1989).

In Europe and elsewhere diflubenzuron is used in a variety of ways not presently permitted in the United States. For example, diflubenzuron and other insect growth regulators are fed as admixtures to rations of chickens, cattle, and swine in order to control fly larvae breeding in their manures, and also as a spray directly on manures prior to disposal (Opdycke et al. 1982b; Opdycke and Menzer 1984; Giga 1987). Diflubenzuron has been administered orally as a bolus to beef cattle for control of face flies (*Musca autumnalis*) and horn flies (*Haematobia irritans*), two serious pests of cattle in North America; immature insects develop in fresh manure on open pasture. A single bolus released diflubenzuron into feces that killed horn and face fly larvae for 8 weeks and remained partially effective for 16 weeks (Scott et al. 1986).

Three diflubenzuron formulations are now in general use: an oil dispersable concentrate, a wettable powder (WP), and granules (Bull 1980; Cunningham 1986; Poplyk 1989). Granular formulations are produced by applying diflubenzuron to sand granules. Since technical diflubenzuron (99.5% pure) is a crystalline material that

is almost insoluble in water (i.e., 0.1 mg/L at 20° C), it is usually dispersed in an organic solvent carrier. Wettable powders (25% active ingredients), however, are dispersed in water for use in many commercial applications; diflubenzuron particle size in WP-25 formulations usually ranges between 2 and 5 microns.

Lethal and Sublethal Effects

General

Diflubenzuron applied to foliage of terrestrial plants tends to remain adsorbed for several weeks with little or no absorption or translocation from plant surfaces; loss is mainly by wind abrasion, rain washing, or shedding of senescent leaves. Among insect species, there is great variability in sensitivity to diflubenzuron. In general, diflubenzuron is toxic to early life stages of insects at concentrations as low as 0.1 mg/kg diet and at topical applications between 0.003 and 0.034 μg per larvae. Among aquatic organisms, early developmental stages of crustaceans and insects are the most sensitive groups tested; adverse effects on growth, survival, reproduction, and behavior occur between 0.062 and 2.0 μg/L. Groups highly resistant to diflubenzuron include the algae, gastropods, fishes, and amphibians. Birds are comparatively resistant: acute oral LD50 values exceed 2,000 mg diflubenzuron per kg body weight (BW), and dietary levels of 4,640 mg/kg ration are tolerated for 8 days. Also, forest birds seem unharmed by recommended diflubenzuron application procedures to control pestiferous insects. No data are available on mammalian wildlife. However, studies with small laboratory animals and domestic livestock suggest a high degree of resistance. No observable adverse effects occur in cows given 0.25 mg/kg BW daily for 4 months, in rabbits given 4 mg/kg BW daily on days 6 to 18 of gestation, in dogs fed diets containing 40 mg/kg for 13 weeks (equivalent to 1.6 mg/kg BW daily), in rats fed diets containing 160 mg/kg for 2 years, and in rabbits and rodents given single oral or dermal doses <2,000 mg/kg BW. All of these points are discussed later.

Terrestrial Plants

There is little to no absorption and translocation of diflubenzuron residues from plant surfaces (Gartrell 1981). Due to its stability and low volatility, diflubenzuron residues adhering to plant surfaces are removed primarily through physical effects such as wind abrasion, rain washing, or the loss of dead leaves (Bull 1980). A greenhouse study with corn (*Zea mays*), soybeans (*Glycine max*), cabbage (*Brassica oleracea capitata*), and apples (*Malus* sp.) showed no significant degradation of diflubenzuron residues in leaves for up to 16 weeks after treatment (Gartrell 1981). In a study with radiolabeled diflubenzuron a single dose applied to a cotton (*Gossypium hirsutum*) leaf showed <5% photodegradation in 4 weeks, <7% absorption in 7 weeks, <50% loss to weathering or volatilization in 4 weeks in samples not exposed to rain, and 77% loss in 3 weeks after a heavy rainfall (Bull and Ivie 1978; Bull 1980). Edible portions of rotational crops treated repeatedly with diflubenzuron at recommended application levels had low, but detectable, residues. Maximum concentrations, in mg/kg DW, were always <0.01 in wheat (*Triticum* spp.), <0.02 in cotton, <0.09 in collards (*Brassica* spp.), and <0.16 in radish (*Raphanus* spp.; Bull and Ivie 1978; Bull 1980).

Foliage of cotton that initially contained 100 mg/kg DW contained about 60 mg/kg after 7 weeks; leaf residues consisted entirely of the parent diflubenzuron (Gartrell 1981).

Diflubenzuron applied topically to lima bean (*Phaseolus lunatus*) foliage was not absorbed by the plant, as expected. Injected diflubenzuron, however, was metabolized, and certain of the metabolites were similar to those isolated from mites (Franklin and Knowles 1981).

Diflubenzuron mixed into compost layers of the cultivated mushroom (*Agaricus bisporus*) at 30 mg/kg compost to control dipteran pests of mushroom resulted in increased yield and size; however, at higher concentrations of 180 mg/kg and 1,080 mg/kg, mushroom yield and number were reduced, and this became more severe over time (White 1986). Frequent applications of diflubenzuron to agricultural soils are not detrimental to nitrogen-fixing bacteria (i.e., *Azotobacter vinelandii*), and high concentrations could stimulate nitrogenase activity in soils. This conclusion is based on a study by Martinez-Toledo et al. (1988) using nonsterile agricultural soils and sterilized soils inoculated with *A. vinelandii*. At diflubenzuron loadings between 100 and 500 mg/kg, all concentrations tested had a stimulatory effect on nitrogen fixation in both soils.

Terrestrial Invertebrates

Diflubenzuron is most toxic to early life stages of some insects at 0.1 mg/kg diet, 0.034 μg per larvae (about 3.1 mg/kg BW), or in combination with various chemicals (Table 3). Some beneficial insects, such as the honey bee (*Apis mellifera*), are adversely affected at dietary concentrations of 1 mg/kg for 12 weeks, 10 mg/kg for 10 weeks, or 59 mg/kg for 10 days (Table 3). At 28 to 56 g/ha (0.025 to 0.05 pounds per acre), diflubenzuron effectively controls mosquitos for 8–15 days (Booth and Ferrell 1977; Schaefer and Dupras 1977), especially organophosphorus insecticide-resistant strains of salt-marsh mosquitos in California (Lee and Scott 1989). Diflubenzuron was also effective in controlling strains of house fly (*Musca domestica*) that were resistant to organochlorine, organophosphorus, carbamate, and pyrethroid insecticides on a United Kingdom pig farm;

Table 3. *Diflubenzuron effects on selected terrestrial invertebrates.*

Organism, dose, and other variables	Effect	Reference[a]
Nematode, *Acrobeloides* sp.		
Fed diet containing 100 mg/kg for 10 days	Population reduction of 97%	1
Boll weevil, *Anthonomus grandis*		
1 μg per female weevil, applied topically	After 8 days, about 62% was not absorbed, 3% was absorbed, and 35% was metabolized and excreted	2
113.4 g/ha, applied five times during winter	Reduced heavy infestations by >70% in upper Gulf coast area of Texas	3
Honey bee, *Apis mellifera*		
Fed sucrose syrup/sugar cake diets containing 0.01, 0.1, 1 or 10 mg/kg for 12 weeks	Adult colony survival reduced at 10 mg/kg; inhibited reproduction at 1 and 10 mg/kg; no measurable effect on survival or reproduction at 0.01 or 0.1 mg/kg	4
Fed diet containing 10 mg/kg for 10 weeks	No reduction in consumption of pollen or in quantity of brood reared, but >50% reduction in amount of sucrose syrup stored	5
Fed sucrose syrup containing 59 mg/kg for 10 days	Inhibited reproduction	6
Fed sucrose syrup containing 60 mg/L and drinking water containing 100 mg/L for 40 days	Treated bees consumed significantly less water and pollen and produced significantly less comb, brood, and new workers	7
German cockroach, *Blattella germanica*		
Nymphs fed diets containing 4, 20, 100, or 500 mg/kg for 4 weeks	None dead at 4 mg/kg, 15% at 20 mg/kg, 88% at 100 mg/kg, and all dead at 500 mg/kg	8
Common green lacewing *Chrysopa carnea*		
0.5 g/L spray	Reduced incubation period, reduced hatch, and reduced survival	9
2.0 g/L spray	No hatch	9
Termite, *Coptotermes heimi*		
Nymphs fed diets containing 100, 500, or 1,000 mg/kg	All dead in 24 days at 100 mg/kg, 20 days at 500 mg/kg, or 16 days at 1,000 mg/kg. Some nymphs developed blister-like swellings on the abdomen and failed to molt into the next instar	10
Mosquito, *Culex pipiens quinquefasciatus*		
Adults fed 500 or 1,000 mg/kg diet for 2 days	At both doses, 40% of eggs failed to hatch or hatched abnormally; at the high dose, ovarian histopathology recorded	11
Cat flea, *Ctenocephalides felis*		
Larvae, held in rearing medium for 5–6 weeks		
90 μg/kg	LC 50, 1.5-day-old larvae	12
2,220 μg/kg	LC 50, 2.5-day-old larvae	12
>100 mg/kg	LC 50, 3.5-day-old larvae	12
Termite, *Heterotermes indicola*		
Nymphs fed diets containing	All dead in 14–16 days at 100–1,000 mg/kg diet	10

Table 3. *Continued.*

Organism, dose, and other variables	Effect	Reference[a]
100, 500, or 1,000 mg/kg feed		
Gypsy moth, *Lymantria dispar*		
100 μg/kg diet	100% lethal to larvae	13
Cabbage moth, *Mamestra brassicae*		
2.2 mg/L spray	LC90, third instar larvae	14
Nematodes, various species		
Fed diet containing 1 mg/kg for 10 days	No effect on reproduction	1
Fed diet containing 10 mg/kg for 10 days	53% population reduction in *Panagrellus redvirus*, and 95% reduction in *Pelodera* sp.	1
American cockroach, *Periplaneta americana*		
Nymphs fed diets containing 100 or 800 mg/kg for 4 weeks	17% dead at low dose and 52% dead at high dose	8
Large white butterfly, *Pieris brassicae*		
0.39 mg/L spray	LC50, third instar larvae	14
Cotton leafworm, *Spodoptera littoralis*, fourth instar larvae, topical application		
3, 10, 30, or 100 ng per larva	Incorporation of N-acetyl glucosamine into chitin was inhibited by 23% at 3 ng per larva, 75% at 10 ng, 90% at 30 ng, and 98% at 100 ng	14
34 ng per larva, equivalent to 3.1 mg/kg BW	LD50, applied in combination with profenofos	15
468 ng per larva, equivalent to 42.5 mg/kg BW	LD50	15
4.3 mg/L, spray	LC90, third instar larvae	14
Termites		
Nymphs, 3 species, given 100–1,000 mg/kg diet	All dead in 14–24 days	10
Adults, 2 species, given 1,000 mg/kg diet	Fecundity reduced and eggs failed to develop	10

[a] 1, Veech 1978; 2, Bull 1980; 3, Cole 1980; 4, Stoner and Wilson 1982; 5, Nation et al. 1986; 6, Muzzarelli 1986; 7, Barker and Waller 1978; 8, Tsuji and Taneike 1988; 9, Zaki and Gesraha 1987; 10, Ahmad et al. 1986; 11, Mittal and Kohli 1988; 12, El-Gazzar et al. 1988; 13, Martinat et al. 1988; 14, Grosscurt et al. 1988; 15, El Saidy et al. 1989.

416 mg/m^2 to slurry pots of pig weaning rooms gave effective control 2–4 weeks after application (Webb and Wildey 1986).

Chemical control of larvae of gypsy moth and other forest-insect defoliators may cause indiscriminate reduction of nontarget arthropods, which, in turn, may affect food resources of forest birds and small mammals. This problem is of special concern in West Virginia, where two species of endangered bats (Indiana bat, *Myotis sodalis*; eastern big-eared bat, *Plecotus phyllotis*) occur in areas threatened by gypsy moth defoliation (Martinat et al. 1988). Diflubenzuron applications, usually at 70 g/ha on 2 consecutive days, controlled gypsy moth larvae and also significantly reduced populations of canopy macrolepidoptera and nonlepidopteran mandibulate herbivores. Sucking herbivorous insects, microlepidop-

tera, and predaceous arthropods, however, were relatively unaffected, which suggests that although diflubenzuron can potentially affect food supply of forest birds and small mammals, these effects are probably minimal (Martinat et al. 1988).

Researchers generally agree that diflubenzuron causes incomplete ecdysis by interfering with chitin synthesis. However, diflubenzuron at lethal concentrations causes an effect in chironomid larvae (*Chironomus decorus, Tanypus grodhausi*) other than inhibition of chitin synthetase, as judged by histopathology of the alimentary canal—especially the ventriculus. Dysfunction of the ventriculus, an organ that normally lacks chitin, results in a general breakdown of the digestive apparatus of exposed chironomid larvae (Pelsue 1985).

Exposure of nematodes and of adults of several insect species, including boll weevil, housefly, and stable fly (*Stomoxys calcitrans*), to diflubenzuron results in deposition of eggs that appear normal but fail to hatch. This effect seems to be due to an ovicidal action and not to sterility of the treated adults, since the larvae appear to undergo normal development within the egg. Secretion of unmetabolized diflubenzuron into the eggs apparently accounts for observed ovicidal effects (Ivie and Wright 1978; Veech 1978; Ivie et al. 1980). Treated female boll weevils began to lay viable eggs 12 days after treatment and became as productive as controls in 24 days; additional treatment is required to maintain a significant suppression of egg hatch (Bull 1980).

Diflubenzuron is the most investigated benzoylphenylurea and has shown excellent potency for controlling mosquitos and certain lepidopterous and coleopterous pests. Some insect species, however, cannot be controlled efficiently by diflubenzuron. For example, the cotton leafworm (*Spodoptera littoralis*) is comparatively resistant because of reduced penetration through the exoskeleton, rapid elimination of unchanged diflubenzuron, and rapid metabolism, which occurs mainly through hydrolysis (El Saidy et al. 1989). To combat *Spodoptera* and other resistant pests, new benzoylphenylurea compounds have been developed, including chlorfluzuaron, teflubenzuron, and hexafluron (El Saidy et al. 1989).

Beneficial insects associated with fruit orchards show different responses to diflubenzuron treatment (Broadbent and Pree 1984). Lacewings (*Chrysopa oculata*) in contact with leaves containing 300 mg/kg DW had reduced survival and inhibited molting of first instar larvae, but the assassin bug (*Acholla multispinosa*) was not affected by contact with treated leaves. Lacewings and other beneficial predator insects fed diflubenzuron-treated two-spotted spider mites (*Tetranychus urticae*) for 3 days showed no adverse effects after 14 days (Broadbent and Pree 1984). Spraying of diflubenzuron at 28 g/ha to control gypsy moth did not affect *Cotesia melanoscela*, a hymenopteran predator of the gypsy moth; however, another natural enemy, a virus, was adversely affected (Webb et al. 1989). Certain arthropod predators were unaffected by diflubenzuron at 70 mg/ha applied four times in 3 weeks to control the boll weevil; these include the convergent lady beetle (*Hippodamia convergens*), the bigeyed bug (*Geocoris punctipes*), and various species of *Coleomegilla, Orius, Nabis,* and *Chrysopa* (Deakle and Bradley 1982).

Diflubenzuron can be either hydrolyzed at the urea bridge or oxidized by ring hydroxylation followed by conjugation. Hydrolytic cleavage seems to be a major route for diflubenzuron metabolism in many insect species (El Saidy et al. 1989). Two-spotted spider mites showed <10% absorption in 96 h of topically applied diflubenzuron. Of the amount absorbed about 27% was metabolized in 96 h to 4-chlorophenyl urea, 2,6-difluorobenzoic acid, 4′-chloroformanilide, 2,6-difluorobenzamide, and other metabolites (Franklin and Knowles 1981). Effects of diflubenzuron were synergized by profenofos (El Saidy et al. 1989) in cotton leafworm 4th instar larvae, and they were antagonized by 20-hydroxyecdysone (Soltani et al. 1987) in yellow mealworm beetle (*Tenebrio molitor*) pupae. More information is needed on interaction effects of diflubenzuron with other chemicals.

Aquatic Organisms

Laboratory Studies

Studies with diflubenzuron and representative aquatic organisms under controlled conditions (Table 4) show several trends:

1. Crustaceans are the most sensitive group of nontarget organisms tested—adverse effects on growth, survival, reproduction, and behavior of copepods, shrimp, daphnids, amphipods, and crabs occur between 0.062 and 2.0 μg/L medium, and early developmental stages were the most vulnerable;
2. Next in sensitivity are aquatic insects, including mayflies, chironomids, caddisflies, and midges—diflubenzuron concentrations between 0.1 and 1.9 μg/L medium produce low emergence and survival;
3. Other groups tested are comparatively resistant, that is, adverse effects occur at <45 μg/L—in fish, for example, death occurred at >33,000 μg/L; and
4. Elevated accumulations occur in aquatic plants during exposure to 100 μg/L and in fish during exposure between 1 and 13 μg/L. All species in these groups, however, seemed unaffected by elevated body burdens, as judged by normal growth and metabolism.

The major degradation products of diflubenzuron in water are 4-chlorophenylurea and 2,6-difluorobenzoic acid (Metcalf et al. 1975; Ivie et al. 1980); these compounds

Table 4. *Diflubenzuron effects on selected aquatic organisms: laboratory studies.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
Algae and macrophytes		
Diatom, *Cyclotella cryptica*		
5,000	No effect on photosynthesis during 14-day exposure	1
Bluegreen alga, *Plectonema boryanum*		
100	Residues, in µg/kg dry weight, during exposure for 4 days were 144,700 at 1 h, 85,700 at 1 day, 56,900 at 2 days, 11,700 at 3 days, and 8,300 at 4 days; *Plectonema* growth rate was unaffected	2
Alga, *Selenastrum capricornutum*		
45	No effect on growth during exposure for 120 h	3
Diatoms, 3 species (*Skeletonema costatum, Thalassiosira nordenskioldi, T. weissflogii*)		
1,000	No effect on photosynthesis during exposure for 11–14 days	1
5,000	Photosynthesis inhibited 70–80% in 11- to 14-day exposure	1
Coelenterata		
Hydra, *Hydra oligactis*		
0.1–0.12 (estimated)	After 24-h exposure, asexual budding rate significantly increased over controls during 20-day posttreatment period; some histopathology. Second generation hydras not significantly different from controls	4
Platyhelminthes		
Planarian, *Dugesia dorotocephala*		
5	No effect on survival, behavior, or asexual reproductive capacity after 24-h exposure	23
Aquatic insects		
Mosquito, *Aedes aegypti*, fourth instar larvae		
20 (equivalent to 0.056 kg/ha)	Fatal to 100% within 24 h, about 50% after 4 days, and <20% after 8 days	5
Mosquito, *Aedes albopictus*		
0.00025	LC30 (24 h), second instar larvae	6
0.0028	Adult emergence inhibited when second instar larvae exposed for 24 h	6
0.025	LC67 (24 h), second instar larvae	6
0.125	Histopathology of cuticle and anal gills in fourth instar larvae after 24-h exposure of third instar larvae	6
0.21	Adult emergence inhibited when third instar larvae exposed for 24 h	6
0.25	LC67 (24 h), third instar larvae	6

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
12.5	No histopathology of fourth instar larvae after 24-h exposure	6
25	LC16 (24 h), fourth instar larvae	6
39.6	Adult emergence inhibited when fourth instar larvae exposed for 24 h	6
Mosquito, *Aedes nigromaculis*		
0.5	LC50 (48 h), larvae	7
Aquatic beetles *Hydrophilus triangularis*		
100	LC50 (48 h), larvae	7
Laccophilus spp.		
250	No deaths of adults in 216 h	7
Thermonectus basillaris		
250	No deaths of adults in 168 h	7
Tropisternus lateralis		
250	No deaths of adults in 48 h	7
Mayfly, *Callibaetis* sp.		
10	LC90 (168 h), nymphs	7
Chironomid, *Chironomus decorus*, fourth instar		
1.9	LC50	8
6.0	LC90	8
Midge, *Chironomus plumosus*		
560	50% of larvae immobilized in 48 h	9,10
Caddisfly, *Clistoronia magnifica*		
0.1	Adult emergence inhibited during 4-week exposure	11
Midge, *Cricotopus* spp.		
1.6	No adult emergence in 96-h exposure	11
4.9	Molting and survival adversely affected during exposure for 96 h	11
Mosquito, *Culex pipiens* exposed as fourth instar larvae for 24 h		
8	50% reduction in adult emergence	12
100	74% reduction in adult emergence	12
1,000	No adult emergence	12
Mosquito, *Culex pipens quinquefasciatus*		
1.0	Fourth instar larval dip had no effect on adult sterility	13
Chironomid, *Glyptotendipes paripes*, fourth instar		
1.8	LC50	8
4.1	LC90	8

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
Midge, *Goeldichironomus holoprasinus*		
10	LC90 (168 h), larvae	7
Dragonflies, *Orthemis* spp., *Pantala* sp.		
50	LC50 (168 h)	7
Blackfly, *Simulium vittatum*, larvae		
80 for 30 min at various water temperatures		
10°C	50% dead in 21 days	14
20°C	53% dead in 13 days	14
25°C	92% dead in 3 days	14
500 for 15 min	98% dead in 18 days at 10.5°C	14
1,000 for 30 min	All dead in 10 days at 15°C	14
Stonefly, *Skwala* sp.		
57,500	LC50 (96 h)	15
Midge, *Tanytarsus dissimilis*		
1.0	LC50, period between second and third instar larvae	3
4.9	Molting and survival adversely affected during 5-day exposure	11
Arachnoids		
Horseshoe crab, *Limulus polyphemus*		
5	Larvae exposed for 24 days showed slight delay in molting at 14 days; survival as in controls	6
50	Larvae exposed for 24 days showed molt rate as in controls, but high mortality immediately after ecdysis; reduced growth of survivors	16
Molluscs		
Clam, *Anodonta cygnea*		
200,000	After exposure for 3 months, all clams survived and appeared healthy. But normal calcification process disrupted on lamellar layer of the shell, producing fragile shell	17
Snail, *Juga plicifera*		
36–45	No effect on survival, growth, or reproduction during 3-week exposure	3,11
Snails, *Physa* spp.		
45	No measurable effect on growth, survival, or reproduction during 3-week exposure	3,11
Crustaceans		
Copepod, *Acartia tonsa*		
Adults exposed following terminal molt		

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in μg/L (ppb)	Effect	Reference[a]
1	Hatch of viable nauplii reduced by 50% after 12-h exposure; no hatch after 36-h exposure. Effect not reversible for at least 30 h after exposure	18
10	Hatch of nauplii reduced by >95% after exposure for 24 h and 100% after 36 h. Effect not reversible for at least 26 h after exposure	18
100	No effect on egg production during 14-day exposure	18
1,000	No adverse effect on survival during exposure for 5 days	18
Brine shrimp, *Artemia salina*		
Adults exposed to 1, 2, 5, or 10	During exposure for 80 days, there was a significant reduction in reproductive lifespan at 2, 5, and 10 μg/L. Nauplii produced viviparously by mated pairs were comparable to controls—except for the 10 μg/L group, which produced fewer nauplii. Cysts produced oviparously by treated pairs, however, had lower mean hatchability	19
Nauplii exposed to 1, 10, or 100	All dead within 30 days in the 100 μg/L group; survival same as controls in 12 days for the 1 and 10 μg/L groups	19
Barnacle, *Balanus eburneus*		
50	Some deaths when exposure exceeds 10 days	20
50–100	Significant acceleration of intermolt cycle at low dose, and among survivors at high dose	21
100	No deaths of adults in 28 days	21
750 or 1,000	High mortality during 10-day exposure; prolonged premolt; histopathology of cuticle-secreting epidermal cells	20
1,000 for 48 h plus clean seawater for 26 days	No deaths of adults	21
1,000 for 72 h plus clean seawater for 25 days	High mortality of adults, especially on days 7–14 postexposure	21
Copepods, 2 species		
100	Negligible mortality in 144 h	7
Blue crab, *Callinectes sapidus*		
1	High survival of megalops	22
3	Low survival of megalops	22
Ostracods, *Cypicerus* sp., *Cypridopsis* sp.		
500	Negligible mortality in 72 h	7
Daphnid, *Daphnia magna*		
0.062	Survival and reproduction adversely affected in full life cycle (21-day exposure)	3
2	All dead within 6 days	11

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
4.4–15	50% immobilized or dead in 48 h	3,9,10,15
Daphnids, 2 spp.		
1.5	LC50 (48 h)	7
Clam shrimp, *Eulimnadia* spp.		
0.15	LC50 (48 h)	7
Copepod, *Eurytemora affinis*		
0.75–1.00	MATC[b]	49
2.2	LC50 (48 h) for nauplii	49
Scud (amphipod), *Gammarus pseudolimnaeus*		
25–45	LC50 (96 h)	9,10,15
1,000	After exposure for 30 min, 22% dead in 55 days at 15°C	14
1,000	After 30-min exposure, 91% dead in 9 days at 25°C	14
Amphipod, *Hyalella azteca*		
1.8	LC50 (96 h)	3
2	LC60 (96 h)	11
1,000	After 30-min exposure, 3–7% dead in 19–21 days at 15°C	14
1,000	After 30-min exposure, 62–99% dead in 7–12 days at 25°C	14
Stone crab, *Menippe mercenaria*		
0.5	LC50 (48 h), larvae	22,24
Copepod, *Mesocyclops thermocyclopoides*		
1–15	Impaired fertility of ovigerous females	25
2–1,000	Prolongation of copepodite stage for 3–4 days followed by death without molting, in most cases. At 125 µg/L and higher, partial molting occurred but all died	25
500	No larval deaths in 48 h	25
1,000	LC50 (48 h) for copepodites. No effect on mating behavior of adults but abnormal ovisac development and decreased fecundity in some females	25
Mysid shrimp, *Mysidopis bahia*		
0.075	Reduction in number of young per female after exposure for 21 days	26
0.075	Reduced survival and reproductive success after exposure for 28 days	24
1.2	LC50 (21 days)	26
1.9	Exposure for 24 h resulted in 65% mortality 3 days after treatment; progeny produced before death had a significantly lower reproduction rate than controls, as did those in the next generation at nanogram/L (ppt) concentrations	27

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
2.0–2.1	LC50 (96 h)	24,26,27,28
Grass shrimp, *Palamonetes pugio*		
0.1–0.5	No effect on duration of molt cycle, but dose-related inhibition of regenerative limb growth noted (EC50 = 0.11 µg/L for left 5th periopod)	29
0.3–0.5	MATC[b]	30
0.3–0.5	Loss of positive phototaxis in embryos exposed for 96 h	31
0.3–1.0	Dose-dependent increase in swimming speed in light-adapted larvae	30
0.65	LC50 (7–14 days) intermolt-molt stage; deaths noted during or immediately after molting	29
<1.0	Almost all larvae that survived to day 15 eventually metamorphosed successfully to postlarvae	32
1.0 (initial), medium aged for 71 days, with or without sediments	No deaths of larvae in 22 days when sediments present; all larvae died within 22 days when sediments absent	34
1.0	Limited vertical migration of larvae	30
1.1	LC50 (96 h), early premolt stage	29
1.4	LC50 (96 h) larvae, 95% confidence interval (CI) 1.27–1.54, for wettable powder (WP-25) in water	32
1.6	LC50 (96 h), postlarvae	33
1.8	LC50 (96 h), larvae, 95% CI for technical grade in acetone is 1.64–2.08	32
2.5	All dead by day 15 regardless of formulation tested	32
2.5–5	Morphological abnormalities, both positive and negative phototaxis suppressed	29
3.4	LC50 (24-h exposure, held until molting complete in 24–48 h)	29
202	LC50 (96 h), adult males and nonovigerous females	33
2,000	Negligible mortality of late premolt stage during exposure for 96 h	29
6,985	LC50 (96 h), adult ovigerous females	33
Crab, *Rithropanopeus harrisii*		
0.05	No effect on positive phototaxis response of stage IV larvae	35
0.1	Reduced positive phototaxis of stage IV larvae	35
0.3–0.5	Increased swimming speed of stage I, II, and III larvae	35
0.5	No adverse effects on larval survival during exposure for 20 days	36
1.0	Decreased larval survival during 20-day exposure	36
10	All larvae died during 32-day exposure in containers without sediments; containers with sediments were no longer toxic after 19 days	34

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
Crab, *Sesarma reticulatum*		
1	No adverse effects on larval survival during 40-day exposure	36
3	Decreased larval survival during 40-day exposure	36
10	All larvae died during 40-day exposure	36
Copepod, *Tigriopus californicus*		
0.1–100	During 72-day exposure, no adverse effects were noted on adult survival and juvenile development at 0.1 µg/L. Reproduction was inhibited at 1 and 5 µg/L. Copepods exposed to 10 or 100 µg/L did not reproduce, were moribund, and had decreased survival.	1
Tadpole shrimp, *Triops longicaudatus*		
0.75	LC40 (24 h)	7
Fiddler crab, *Uca pugilator*		
Juveniles exposed for 24 h once a week for 10 weeks, then held in clean seawater for an additional 14 weeks		
0.2	No adverse effects on survival or ability to escape from test containers	37
2	No effect on survival, but reduced ability to escape from container	37
20	All died in 23 weeks; reduced mobility prior to death	37
200	All dead in 8 weeks; most deaths occurred in first 4 weeks	37
Adults exposed to 0.5, 5, or 50 after multiple autotomy of one chela and 5 walking legs	Continuous exposure for 18 days produced a dose-dependent retardation of regeneration and deaths during molt at 5 and 50 µg/L. The presence of sediment in test containers lessened effects, but did not eliminate them	38
Adults exposed for 13 weeks		
0.5–50	Some reduction in number of burrows dug at 15 and 60 min after exposure	39
Unknown	Burrowing activity normal on sediments containing 1 mg/kg	39
Fish		
Mummichog, *Fundulus heteroclitus*		
29,800	No deaths in 96 h	40
33,000–255,000	LC50 (96 h)	5,40
Mosquitofish, *Gambusia affinis*		
Unknown	Fish exposed to radiolabeled diflubenzuron for 33 days contained about 6% of the parent diflubenzuron vs. 54% for alga (*Oedogonium cardiacum*), 82% for snail (*Physa* sp.), and	42

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
	94% for larvae of mosquito (*Culex pipiens quinquefasciatus*)	
200 for 8 days followed by 300 until day 14	Fish were 2.5× more hyperactive than controls within 2 days, four times more during days 4–8, and no different from controls at day 14	43
1,000	No deaths in 10 days	7
Brown bullhead, *Ictalurus nebulosus*		
13.2 in pond surface layer 1 h after treatment, <0.2 after 14 days	Maximum concentrations, in µg/kg whole body fresh weight (FW), were 387 at day 1, 190 at day 2, 42 at day 4, and ND at day 7	44
Channel catfish, *Ictalurus punctatus*		
Unknown	Runoff from soil containing 0.55 mg diflubenzuron/kg at start produced maximum residues during 28 days, in µg/kg FW, of 4 in muscle and 10 in viscera	2
>100,000	LC50 (96 h)	10,15
370,000	LC50 (96 h)	9
Bluegill, *Lepomis macrochirus*		
1–10	Bioconcentration factor of 13–20 times after 24-h exposure	45
10	After exposure for 24 h, residues 24 and 48 h later were 848 and 8 µg/kg FW whole fish (less tail and viscera)	45
200 (initial); diflubenzuron concentrations ranged from 1.3 to 5.1 between 3 h and 19 days; for 4-chlorophenylurea they were 0.6–3.1, and for 4-chloroaniline they were <0.1–0.4	Maximum concentrations in µg/kg whole fish FW, for diflubenzuron were 119 at 3 h, 812 at 1 day, 55 at 5 days, 196 at 12 days, and 86 at 19 days. For the metabolite 4-chlorophenylurea, these values were 1.6 at 3 h, 8 at 1 day, 40 at 50 days, and 33 at 19 days; and for 4-chloroaniline, 0.8 at 3 h, 2.1 at 1 day, and 1.1–1.3 for days 5–19	46
135,000–660,000	LC50 (96 h)	5,9
Cutthroat trout, *Oncorhynchus clarki*		
57,000–75,000	LC50 (96 h)	10,15
Coho salmon, *Oncorhynchus kisutch*		
150,000	No deaths in 96 h	47
1,000,000	No deaths in 96 h after 15-min exposure	47
Rainbow trout, *Oncorhynchus mykiss*		
29–300	No adverse effects on eyed eggs or fingerlings in 30-day flowthrough exposure	10
625–10,000	Dose-dependent decrease in serum glutamate oxalacetate transaminase (GOT) activity in 96 h, but values overlapped normal GOT range from this hatchery	50
150,000	No deaths in 96 h	47

Table 4. *Continued.*

Taxonomic group, organism, and concentration in medium in µg/L (ppb)	Effect	Reference[a]
240,000 (95% CI 201,000–286,000)	LC50 (96 h)	9,10
1,000,000	No deaths in 96 h after 15-min exposure	47
Yellow perch, *Perca flavescens*		
>50,000	LC50 (96 h)	15
Fathead minnow, *Pimephales promelas*		
36–45	Embryo-larval exposure for 30 days had no effect on survival, egg hatch, or growth	3,11
>100,000	LC50 (96 h)	10,15
430,000	LC50 (96 h)	9
White crappie, *Pomoxis annularis*		
5 (nominal), 3.3 (measured), 0.4 after 5 weeks	Whole body residues, in µg/kg FW, were 133 at 1 day, 355 at 4 days, 197 at 14 days, and 62 at 21 days	48
10	Fish exposed for 24 h in uncontaminated media contained 822 µg/kg FW whole fish less tail and viscera. Exposure for 48 or 72 h plus 24 h in uncontaminated media produced residues of 533 and 630 µg/kg FW	45
Atlantic salmon, *Salmo salar*		
10	Avoided medium when given choice in 10-min trials	41
>50,000	LC50 (96 h)	15
Brook trout, *Salvelinus fontinalis*		
>50,000	LC50 (96 h)	15

[a] 1, Antia et al. 1985; 2, Booth and Ferrell 1977; 3, Hansen and Garton 1982; 4, Kalafatic and Znidaric 1987; 5, Madder and Lockhart 1980; 6, Ho et al. 1987; 7, Miura and Takahashi 1974; 8, Ali and Lord 1980b; 9, Julin and Sanders 1978; 10, Johnson and Finley 1980; 11, Nebeker et al. 1983; 12, Kelada et al. 1980; 13, Mittal and Kohli 1988; 14, Rodrigues and Kaushik 1986; 15, Mayer and Ellersieck 1986; 16, Weis and Ma 1987; 17, Machado et al. 1990; 18, Tester and Costlow 1981; 19, Cunningham 1976; 20, Gulka et al. 1982; 21, Gulka et al. 1980; 22, Costlow 1979; 23, Levy and Miller 1978; 24, Nimmo et al. 1981; 25, Rao and Paul 1988; 26, Nimmo et al. 1979; 27, Nimmo et al. 1980; 28, Mayer 1987; 29, Touart and Rao 1987; 30, Wilson et al. 1987; 31, Wilson et al. 1985; 32, Wilson and Costlow 1986; 33, Wilson and Costlow 1987, 34, Cunningham et al. 1987; 35, Forward and Costlow 1978; 36, Christiansen et al. 1978; 37, Cunningham and Myers 1987; 38, Weis et al. 1987; 39, Weis and Perlmutter 1987; 40, Lee and Scott 1989; 41, Granett et al. 1978; 42, Metcalf et al. 1975; 43, Ellgaard et al. 1979; 44, Colwell and Schaefer 1980; 45, Schaefer et al. 1979; 46, Schaefer et al. 1980; 47, McKague and Pridmore 1978; 48,Apperson et al. 1978; 49, Savitz 1991; 50, Madder and Lockhart 1978.

[b] Maximum acceptable toxicant concentration. Lower value in each pair indicates highest concentration tested producing no measurable effect on growth, survival, reproduction, or metabolism during chronic exposure; higher value indicates lowest concentration tested producing a measurable effect.

are less toxic to aquatic organisms than the parent chemical (Julin and Sanders 1978; Schaefer et al. 1979, 1980; Gattavecchia et al. 1981). A minor metabolite, 4-chloroaniline, which is classified as a mutagen by the National Cancer Institute and the Cancer Assessment group of the U.S. Environmental Protection Agency (Schaefer et al. 1980), is significantly more toxic to fish and *Euglena gracilis* than is diflubenzuron. For example, LC50 (96 h) values for 4-chloroaniline and four species of freshwater teleosts are 16 to 56 times lower than comparable data for diflubenzuron, but 4-chloroaniline is 76 times less toxic to *Chironomus* midge larvae in 48 h than diflubenzuron (Julin and Sanders 1978). There is a dose-dependent effect of 4-chloroaniline on *Euglena* growth inhibition and glycine metabolism in the range of 1–200 mg/L during exposure for 30 h (Gattavecchia et al. 1981).

The most sensitive organism to 4-chloroaniline is bluegill (*Lepomis macrochirus*) with an LC50 (96 h) value of 2.3 mg/L (Julin and Sanders 1978). It is unlikely, however, that this concentration will be encountered under current diflubenzuron application practices.

Diflubenzuron inhibits several enzyme systems in crab and insect larvae, resulting in disrupted glucose metabolism, reduced N-acetylglucosamine incorporation into cuticle, and ultrastructural deformities of chitinous components of the cuticle (Christiansen and Costlow 1982; Christiansen et al. 1984; Christiansen 1986). Specifically, diflubenzuron inhibits chitin synthetase, a magnesium-requiring enzyme that catalyzes the transfer of N-acetyl-D-glucosamine to chitin; the final result is relatively large accumulations of N-acetylglucosamines (Horst 1981; Machado et al. 1990).

Diflubenzuron acts specifically on insects and crustaceans as a larvicide by interfering with chitin deposition into cuticles during juvenile development through ecdysis (Horst 1981; Antia et al. 1985; Cunningham 1986; Machado et al. 1990). The biosynthesis of chitin in arthropods is under hormonal control. Arthropods increase in size by resorbing a portion of the shell and initiating the secretion of a new exoskeleton under the old cuticle. At this time, chitin synthesis is maximal. After completion of about half the new shell, molting occurs, the old shell is discarded, and the new shell is synthesized. Diflubenzuron exposure produces disturbances in the cuticular structure, weakening the cuticle so that it fails mechanically during ecdysis of insects and crustaceans. In general, treated larvae appear healthy during the entire intermolt period until molting commences, at which time many larvae are unable to cast their molts completely and die within a few hours. Several genera of diatoms, including *Thalassiosira* and *Skeletonema*, produce up to 33% of their biomass as chitin. These diatoms synthesize chitin strands that extend outside their frustules to increase buoyancy (Montgomery et al. 1990). Chitin-producing diatoms, as well as nonchitanaceous diatoms, seem unaffected at elevated concentrations of 1 mg/L for periods up to 14 days (Antia et al. 1985). Some species of algae, especially *Plectonema boryanum*, are reported to efficiently degrade diflubenzuron (Schooley and Quistad 1979), but this requires verification.

Studies with laboratory stream communities dosed for 5 months confirm that insects and crustaceans are the most severely affected groups; adverse effects occur in the range 1.0–1.1 μg diflubenzuron per L. Fish and molluscs, however, show no adverse effects at 45 μg/L (Hansen and Garton 1982). Freshwater clams (*Anodonta cygnea*) exposed to high concentrations of diflubenzuron for lengthy periods may experience blocked polycondensation reactions to chitin chains in the outer mantle epithelium secretory cells, producing unstabilized chitin and increasing shell fragility. On this basis the comparatively resistant burrowing bivalve molluscs may be at risk if exposed over several calcification periods (Machado et al. 1990).

Fish accumulated diflubenzuron from water up to 160 times water levels, but tissue concentrations during exposure declined steadily over time (Schaefer et al. 1980).

Exposure of *Aedes albopictus*, a mosquito vector of dengue and encephalitis in Taiwan, for 24 h to 0.00025 to 25 μg/L diflubenzuron resulted in dose-dependent aberrations in larvae, pupae, and adults (Ho et al. 1987; Table 4). In general, most treated second and third instar *Aedes* larvae died during molting, while most fourth instar larvae developed abnormally (Ho et al. 1987). Unfortunately, levels of diflubenzuron used to control saltwater mosquitos and other insects are also toxic to zoeal stages of crustaceans (Costlow 1979) and adversely affect growth and reproduction of adults (Muzzarelli 1986). Treated larvae of estuarine crustaceans are characterized by the following: histological alterations in the cuticular layers of the exoskeleton at concentrations as low as 1 μg/L, higher mortality associated with molting and gross morphological deformities at concentrations as low as 0.5 μg/L, and behavioral modifications at concentrations as low as 0.1 μg/L (Cunningham 1986; Table 4). Behavioral effects in fiddler crabs (*Uca pugilator*) were the most sensitive indicator of diflubenzuron stress, and these effects may influence the ability of juvenile crabs to avoid predation, construct burrows, or feed adequately in nature (Cunningham and Myers 1987). Behavioral effects on cladocerans that may result in latent mortality include reduced filter feeding rates, reduced body movements, and inability to exhibit positive phototaxis, a characteristic of untreated individuals (Cunningham 1986; Table 4). Shrimp larvae exposed to ≥2.5 μg/L will not undergo daily vertical migration, and those exposed to 1 μg/L undergo only limited migration, which could affect horizontal transport and dispersal of populations and reduce recruitment to benthopelagic adult populations (Wilson et al. 1987). In addition to its inhibitory effect on cuticle synthesis diflubenzuron affects hormone balance by delaying or arresting the molt cycle, and it inhibits limb regeneration by inhibiting mitosis and differentation (Touart and Rao 1987). Regenerated limbs of diflubenzuron-stressed crabs that survived ecdysis had lesions in the form of black areas in which the cuticle was improperly developed (Weis et al. 1987). Also, diflubenzuron caused a reduction in metabolism of beta-ecdysone in larval insects, leading to an excess of this molting hormone in the tissues. Treatment of decapod crustaceans with ecdysones frequently causes high mortality and molt acceleration (Gulka et al. 1982).

Toxicity and persistence of diflubenzuron in aquatic environments depend on formulation, frequency of application, quantity of organic matter, sediment type, and water pH and temperature. Biological variables are more important than physical variables in assessing difluben-

zuron toxicity, especially the age of the test organism and frequency and synchrony of molting during the exposure period (Cunningham 1986). Crustaceans and other organisms that molt do not demonstrate a typical survival dose-response curve against diflubenzuron because death occurs only when molting is blocked (Nebeker et al. 1983; Cunningham 1986; Cunningham and Myers 1987; Wilson and Costlow 1987). In general, the most sensitive species had comparatively short larval or nymphal periods, and the organism molted frequently (Rodrigues and Kaushik 1986). Susceptible species include mayflies (*Leptophlebia* sp., *Baetis pygmaeus*), while more-resistant species include the stonefly (*Paragnetina media*) and caddisfly (*Hydropsyche bettani*). Amphipods were especially sensitive at 25° C, but not at 10°, 15°, or 20° C (Rodrigues and Kaushik 1986).

Mortality patterns of megalops larvae of blue crab (*Callinectes sapidus*) were elevated at higher temperatures but were seemingly unaffected by water salinity (Costlow 1979). In studies on larvae of black fly (*Simulium vittatum*), diflubenzuron was more effective against earlier larval instar stages than later ones; against rapidly growing larvae than starved, slow-growing larvae; and at 25° than at 20° C (Rodrigues and Kaushik 1986). Among diflubenzuron-stressed barnacles (*Balanus eburneus*), mortality was higher in fed groups than in starved groups, perhaps due to an increased uptake from contaminated food or to an increased molting rate due to feeding (Gulka et al. 1980). Increased fragility of cast exuviae from diflubenzuron-treated barnacles suggests mechanical weakening of the cuticle due to a decrease in chitin content (Gulka et al. 1982).

Field Studies

Field use of diflubenzuron in aquatic habitats for control of pestiferous insects also affects other species (Table 5). Diflubenzuron applications in marshes, ponds, streams, lakes, and rice fields routinely cause population reductions—sometimes irreversible—in many species of nontarget organisms, especially crustaceans and aquatic insects. Taxonomic groups that seem comparatively tolerant to diflubenzuron include algae, turbellarians, rotifers, aquatic beetles, molluscs, annelid worms, ostracods, and fish (Table 5). Following multiple applications to lake and pond ecosystems, diflubenzuron was not measurable in water, sediment, and aquatic vegetation after several days (Booth and Ferrell 1977). Algae (*Plectonema boryanum*) reportedly degrade 80% of absorbed diflubenzuron in 1 h, primarily to 4-chlorophenylurea and 4-chloroaniline (Booth and Ferrell 1977).

Most authorities agree on four points:

1. Rates as low as 28 to 56 g diflubenzuron per surface ha (0.025 to 0.05 pounds per surface acre), or 2.5 to 16 μg/L, are highly effective against pestiferous dipterans, including many species of chaoborids, chironomids, and culicids (Mulla et al. 1975; Julin and Sanders 1978; Ali and Lord 1980a, 1980b; Cunningham 1986; Ali et al. 1988);
2. These same dosages suppress nontarget populations of cladocerans, copepods, mayfly nymphs, corixids, and springtails (Miura and Takahashi 1975; Mulla et al. 1975; Booth and Ferrell 1977; Julin and Sanders 1978; Ali and Lord 1980a; Cunningham 1986; Ali et al. 1988);
3. Moderately resistant to diflubenzuron are larvae of diving beetles, dragonfly adults and naiads, ostracods (*Cypericercus, Cyprinotus*), backswimmers, and water boatmen; highly resistant species include mosquitofish (*Gambusia affinis*), frogs and toads, snails, and algae (Miura and Takahashi 1974; Mulla et al. 1975; Nimmo et al. 1980); and
4. All populations of survivors begin to recover within days or weeks, and recovery is usually complete within 80 days after the last treatment (Mulla et al. 1975; Booth and Ferrell 1977; Ali and Lord 1980a; Nimmo et al. 1980; Cunningham 1986).

Unlike laboratory studies, diflubenzuron does not bioaccumulate markedly in fish or biomagnify through food chains, although altered feeding habits may occur. Under field conditions, marsh or pond sediments usually contain <50 μg/kg FW. This concentration presents negligible risk to channel catfish (*Ictalurus punctatus*) over a 28-day period, suggesting little hazard to catfish during multiple mosquito control applications of diflubenzuron (Booth and Ferrell 1977). Bioaccumulation of diflubenzuron from marsh applications are minimal, as judged by results of uptake studies using marsh sediments containing 550 μg/kg; maximum residues in fish tissues after 3 days were 4 μg/kg FW in muscle and 10 μg/kg DW in viscera (Schooley and Quistad 1979). Diflubenzuron residues are moderately persistent in algae, snails, saltmarsh caterpillars (*Estigmene acrea*), and mosquito larvae but are not biomagnified in food chains ending in fish (Schooley and Quistad 1979).

Maximum diflubenzuron concentrations range from 50 to 720 μg/kg FW in whole body of three species of freshwater teleosts exposed to water treated as many as eight times with 135 g/ha (Gartrell 1981). Feeding habits of freshwater fishes change in ponds showing marked reductions (94–99%) in copepod and cladoceran populations after diflubenzuron treatment (Colwell and Schaefer 1980), perhaps due to availability of various food items. In one study, black crappie (*Pomoxis nigromaculatus*) and brown bullhead (*Ictalurus nebulosus*) altered their diets for 1 month after treatment, eating about three times more insects and ostracods, and almost no cladocerans and copepods—usually major items—than before treatment (Colwell and Schaefer 1980).

Although diflubenzuron is not sprayed directly on fresh waters in gypsy moth control, aerial spraying of

Table 5. *Diflubenzuron effects on selected aquatic organisms: field studies.*

Ecosystem, dose, and other variables	Effect	Reference[a]
Coastal marsh, Louisiana		
6 applications, each of 28 g/ha, over 18-month period	Severe reduction in populations of amphipods, dragonfly naiads, corixid nymphs, and some adult beetles. Increased populations of snails, aquatic insect adults, and two species of fish. No change in 27 taxa. Results confounded by severe drought in experimental and control areas	1,2
Farm pond		
2.5, 5, or 10 μg/L; single application	Inhibited adult emergence by 95–100% of a gnat (*Chaoborus astictopus*), 2 to 7 days after treatment. Crustacean zooplankton suppressed at all treatment levels, especially cladocerans and copepods. Rotifers and algae were not affected. Bluegills that fed predominantly on cladocerans and copepods switched to chironomid midges and terrestrial insects after treatment, with no adverse effects.	3
Laboratory stream communities		
0.1, 1, 10, or 50 μg/L for 5 months	Aquatic insect populations were the most sensitive group, especially mayflys, stoneflys, and dipterans. These, and other invertebrates, showed rapid and permanent reductions in biomass and diversity at 1.0 μg/L and higher. Diversity showed an apparent dose-response relation, with no effect at 0.1 μg/L, intermediate reductions at 1 μg/L, and maximal reductions at 10 and 50 μg/L	4
Lake		
110 g/ha (3.7 μg/L), or 220 g/ha (7.4 μg/L); single application	At low dose, amphipods (*Hyalella azteca*) had 97% population reduction that remained depressed; temporary reduction in cladoceran and copepod populations. At high dose, marked population reductions in cladocerans, copepods, and ostracods (*Cyrinofus* sp.). Oligochaete worms were tolerant to both doses	5
110–280 g/ha	Effectively suppressed adult emergence of nuisance midges (*Tanytarsus, Procladius*) for up to 2 weeks; ineffective against a more pestiferous midge species (*Chironomus decorus*)	6
156 g/ha to lake surface, equivalent to 12 μg/L on April 26 and again on August 24	After first treatment, reduction within 1 week of three species of cladocerans (*Daphnia laevis, Ceriodaphnia* sp., *Bosmina longirostus*), and two species of copepods (*Cyclops* sp., *Diaptomus* sp.). No recovery of *Daphnia* and *Ceriodaphnia* for 6 months, but *Bosmina* reappeared 11 weeks later. *Diaptomus* was depleted for 4 months, but *Cyclops* recovered in 6–7 weeks. The amphipod *Hyallela azteca* was eliminated within 4 weeks, and no recolonization was evident after 6 months. No adverse effects on oligochaetes,	7

Table 5. *Continued.*

Ecosystem, dose, and other variables	Effect	Reference[a]
	snails (*Physa* sp.), or ostracods (*Cyprodopsis* sp.). After second treatment, temporary reduction in *Cyclops* and *Bosmina*, and no significant effects on ostracods, snails, or worms	
Pasture pond		
280 g/ha, single application	Controlled pasture mosquitos, *Aedes nigromaculis* and *A. melanimon*, and caused temporary reductions of cladoceran and mayfly nymph populations. Many cladocerans and mayflys died during the posttreatment ecdysis, characteristically with signs of incomplete cleavage of the middorsal ecdysial suture. More-tolerant groups included corixid and notonectid nymphs, and adult aquatic beetles. No effects on the most tolerant groups: turbellerians (*Mesotoma, Bothromestoma*), rotifers (*Asplanchna*), ostracods, algae, and spiders (*Pardosa* spp., *Lycosa* spp.)	8
Pond		
13.2 μg/L in pond surface layer 1 h after treatment; <0.2 μg/L after 14 days	Residues, in μg/kg whole body FW, in black crappie (*Pomoxis nigromaculatus*) were 426 at day 1, 194 at day 2, 56 at day 4, and not detectable at day 7	9
16 μg/L (estimated from application of 56 g/ha)	Caused declines in third and fourth instar larvae *Culex tarsalis* mosquito 2–8 days after treatment, but not at 11 days	10
80 μg/L (estimated from application of 280 g/ha)	Adult *C. tarsalis* emergence from treated larvae almost completely inhibited for at least 11 days posttreatment	10
Rice field, flooded		
1.1–28 g/ha	100% control of massive rice field populations of fourth instar larvae of the mosquito *Psorophora columbiae* 3–5 days after treatment. Significant reductions in certain nontarget aquatic insect populations	11
About 1,000 μg/L (as judged by 280 g/ha in rice field water 10 cm deep)	Significant reductions in immature populations of the rice water weevil (*Lissorhopterus oryzophilus*) 4–5 days after rice emergence in a continuously flooded field	12
About 1,500 μg/L (420 g/ha)	*Lissorhopterus* population reduced 75% when applied 7 days after rice emergence	12
River		
1,250 μg/L added for 1 h to control simuliid flies	After initial depression, target Diptera (flies), including Simuliidae, increased 4 to 40 times over pretreatment levels after 3–4 weeks, suggesting that one-time applications are useless. No adverse effects on adults and fry 3–4 weeks after exposure of dace (*Phoxinus lagowski*) and minnow (*Leuciscus hakonensis*)	13

[a] 1, Farlow 1976; 2, Farlow et al. 1978; 3, Apperson et al. 1978; 4, Hansen and Garton 1982; 5, Ali and Mulla 1978a; 6, Johnson and Mulla 1981; 7, Ali and Mulla 1978; 8, Miura and Takahashi 1975; 9, Colwell and Schaefer 1980; 10, Mulla et al. 1975; 11, Steelman et al. 1975; 12, Smith et al. 1988; 13, Satake and Yasuno 1987.

large forest tracts may result in exposure of streams by way of leaf litter (Swift et al. 1988a). Residual diflubenzuron was present for at least 4 months on leaves submerged in flowing water, and it was toxic to various invertebrates. For example, treated leaves of the tulip poplar (*Liriodendron tulipifera*) that contain 10 mg diflubenzuron per m^2 after 4 months of submersion produce adverse effects on survival and growth when fed to craneflies (*Tipula abdominalis, Platycentropus radiatus*; Swift et al. 1988b). The effects of diflubenzuron on leaf-litter processing rates in streams is unresolved and merits additional research (Swift et al. 1988a, 1988b).

Birds

Birds are comparatively resistant to diflubenzuron, as judged by the ability of the mallard (*Anas platyrhynchos*) to tolerate single oral doses up to 2,000 mg/kg BW or dietary loadings up to 4,640 mg/kg ration for 8 days (Table 6). Poisoning of insectivorous birds by diflubenzuron, after spraying in orchards as recommended, is highly improbable (Muzzarelli 1986). This conclusion is based on the maximum possible daily intake of insects by wild nestlings (15 mg/kg BW in Great tit, *Parus major*; 10 mg/kg BW in tree sparrow, *Passer montanus*), on a maximum whole body loading of 0.5 mg diflubenzuron per kg FW in insect prey, and on observations of normal growth and subsequent breeding of nestlings in orchards sprayed with diflubenzuron (Muzzarelli 1986).

Despite the apparent absence of direct effects in forest birds, the widespread use of diflubenzuron in the suppression of forest insect defoliators may lead to potentially harmful effects by reducing populations of immature lepidoptera and other mandibulate herbivorous insects upon which they feed. All field evidence collected to date, however, is either inconclusive or negative. In one study, 70.75 g diflubenzuron per ha was applied to an oak forest (*Quercus rubra, Q. velutina, Q. prinus*) in West Virginia to control first and second instars of gypsy moths (Martinat et al. 1987; Table 6). The maximum diflubenzuron residue recorded in a wide variety of canopy forager birds (blue-gray gnatcatcher, *Polioptila caerulea*; great crested flycatcher, *Myiarchus crinitus*; eastern wood-pewee, *Contopus virens*; black-capped chickadee, *Parus atricapillus*; tufted titmouse, *Parus biocolor*; red-eyed vireo, *Vireo olivaceus*; warblers, *Dendroica* spp.; scarlet tanager, *Piranga olivacea*) was 0.21 mg/kg whole body FW. A similar value, 0.20 mg/kg whole body FW, was recorded in ground or low foragers, including wood thrush (*Hylocichla mustelina*); ovenbird (*Seiurus aurocapillus*); rufous-sided towhee (*Pipilo erythrophthalmus*); indigo bunting (*Passerina cyanea*); song sparrow (*Melospiza melodia*), and chipping sparrow (*Spizella passerina*; Martinat et al. 1987).

In another study as much as 280 mg diflubenzuron per ha applied to control the Douglas-fir tussock moth (*Orgyia pseudotsugata*), an important defoliator of true firs (*Abies* spp.) and Douglas-fir (*Pseudotsuga menziesii*) in western North America, had no adverse effects on forest birds, as judged by population censuses, nesting studies, and bird behavior (Richmond et al. 1979; Table 6).

Domestic chickens (*Gallus* spp.) metabolize diflubenzuron to a greater extent than insects, but less than rodents and ruminants. The main pathway of diflubenzuron degradation in chickens is through cleavage of the urea bridge, whereas rats and cows tend to hydroxylate and conjugate the parent molecule (Opdycke and Menzer 1984). Metabolism studies in chickens showed that major residues in tissues and eggs were unchanged diflubenzuron and 4-chlorophenylurea; also present were 2,6-difluorobenzoic acid and 4-chloroaniline (Gartrell 1981). Metabolites in chicken excreta included 4-chlorophenylurea, 4-chloroaniline, 2,6-difluorobenzamide, 2,6-difluorobenzoic acid, and several unidentified compounds (Opdycke and Menzer 1984). At high dietary loadings of 50–500 mg/kg ration, diflubenzuron accumulates in fat, egg, and muscle tissues of chickens; however, excretion is rapid, and residues are usually negligible after 5 weeks on a clean diet (Table 6). Diflubenzuron fed at levels up to 250 mg/kg ration to male broiler chickens for 98 days had no effect on hyaluronic acid (HA) concentration in the combs and wattles (Crookshank et al. 1978). Both chitin and HA are polysaccharides and have a common biochemical precursor, uridine diphospho N-acetylglucosamine (UDPAGA; Crookshank et al. 1978), which is used in the synthesis of chitin by insects and in the production of HA by vertebrates. Since diflubenzuron interferes with the incorporation of UDPAGA into chitin by insects but not with HA production, it would seem that diflubenzuron is relatively harmless to birds; however, more research is needed for verification.

Intraspecies differences in diflubenzuron metabolism are reported for domestic chickens. The White Leghorn breed, for example, produced eggs with significantly higher residues than other breeds tested after 3 weeks on a diet containing 50 mg diflubenzuron per kg, and it had elevated concentrations in fat tissues after 15 weeks on a 10 mg/kg diet (Opdycke et al. 1982b; Opdycke and Menzer 1984). In chickens, diflubenzuron is usually eliminated more rapidly in feces than in eggs, but in the White Leghorn breed the major route of elimination is via egg production. The White Leghorn breed also differed significantly from the Rhode Island Red/Barred Plymouth Rock (RIR/BPR) breed in ability to metabolize diflubenzuron administered orally or intravenously (Table 6). White Leghorn chickens accumulated diflubenzuron to a greater extent than RIR/BPR chickens, and they retained residues for longer periods. Also, White Leghorn chickens produced a higher percentage and greater number of diflubenzuron metabolites in their excreta than other breeds tested (Opdycke et al. 1982b).

Table 6. *Diflubenzuron effects on selected birds.*

Species, route of administration, dose, and other variables	Effect	Reference[a]
Mallard, *Anas platyrhynchos*		
Oral, single dose, 2,000 mg/kg body weight (BW)	Insufficient to kill 50%; anorexia observed on day after treatment	1
Dietary, 4,640 mg/kg ration	Insufficient to kill 50% in 8 days	2
Forest birds		
From oaks (*Quercus* spp.) forest sprayed aerially with 70.75 g diflubenzuron/ha to control gypsy moth instars; samples collected 3 days prior to spraying, and up to 21 days after spraying	Maximum concentrations, in mg/kg fresh weight (FW) whole body, were 0.21 in canopy birds 3 days postspray (0.09 at day 21); 0.20 in understory birds 1 day postspray, and non-detectable (ND) at day 21; 0.45 in foliage 1 day postspray (0.18 at day 21); 0.49 in foliage arthropods at day 3, and 0.1 at day 21; ND in litter at all times; 0.11 in litter arthropods at day 10 and 0.03 at day 21. Controls, in all cases, contained <0.03, except litter arthropods, which contained 0.06 mg/kg	3
Fir and Douglas-fir (*Abies* sp., *Pseudotsuga menziesii*) forest sprayed aerially with 140 or 280 mg/ha to control Douglas-fir tussock moth; effects evaluated in year of spraying and 1 year later	No significant changes in species diversity, brain cholinesterase activity, survival, morbidity, or behavior at either dose. Significant increases in total breeding pairs noted 1 year later in Townsend's warbler (*Dendroica townsendi*), MaGillivray's warbler (*Oporornis tolmiei*), and mountain chickadee (*Parus gambeli*). Some reductions in populations of warbling vireo (*Vireo gilvus*), golden-crowned kinglet (*Regulus satrapa*), and lazuli bunting (*Passerina amoena*), but all differences were attributed to biological variability rather than to insecticide effects	4
Domestic chicken, *Gallus* spp.		
Intravenous injection		
1 mg/kg BW, white leghorn breed, single dose	Half-time (Tb 1/2) persistence in blood of 14.7 h; 12% of dose excreted in 24 h	5
1 mg/kg BW, Rhode Island Red/Barred Plymouth Rock breed (RIR/BPR), single dose	Tb 1/2 of 8.4 h in blood; 29% of dose excreted in 24 h	5
Oral route		
5 mg/kg BW, white leghorn breed, single dose	Maximum residues after dosing, in mg/kg FW, were 0.25 in egg, 0.4 in eggshell, 0.19 in kidney, and 0.16 in ovary. Excretion of 50% in 8–12 h	5,6
5 mg/kg BW, RIR/BPR breed, single dose	Maximum residues after dosing were 0.14 mg/kg FW in eggs and ND in eggshell, kidney, and ovary. Excretion of 51% in 30–36 h and 82–91% in 13 days	5,6
White leghorn and RIR/BPR strains given 5 mg/kg BW daily for 11 days; residues measured in egg during dosing and for 10 days after dosing	Residues, in mg/kg FW egg, for white leghorn strain were highest at days 9 (3.5) and 11 (2.6). Values were 0.04 at day 20, and ND at day 21. Residues in RIR/BPR were lower: 1.7 at day 9, 1.1 at day 11, 0.02 at day 20, and ND at day 21. Tb 1/2 for egg residues ranged between 34 and 38 h	5

Table 6. *Continued.*

Species, route of administration, dose, and other variables	Effect	Reference[a]
Dietary route		
0.05 mg/kg for 28 days	Fat contained 0.018 mg/kg FW at 28 days and <0.0006 mg/kg 7 days after withdrawal	7
0.5 mg/kg for 28 days	Fat contained 0.033 mg/kg FW at 28 days and less than 0.005 mg/kg 7 days after withdrawal	7
1.6 mg/kg for 3 weeks	Minor effects on larvae of house fly (*Musca domestica*) in manure; egg residue of 0.05 mg/kg FW	8
3.1 mg/kg for 3 weeks	Killed 85% of fly larvae in manure; egg residue of 0.25 mg/kg FW	8
5 mg/kg for 28 days	Fat contained 1.16 mg/kg FW at 28 days and <0.032 mg/kg 7–14 days after withdrawal	7
6.2 mg/kg for 3 weeks	Complete inhibition of fly larvae in manure; egg residue of 0.55 mg/kg FW	8
12.5, 25, or 50 mg/kg for 3 weeks	All diets completely inhibited fly larval development in manure; white egg residues, in mg/kg FW, were 1.0 for 12.5 mg/kg diet, 2.1 for 25 group, and 2.9 for the 50 mg/kg group; residues in brown eggs were half those of white eggs	8
Mature white leghorn hens fed diets containing 10, 50, 100, or 500 mg diflubenzuron/kg for 8 weeks	No adverse effects of any diet on feed consumption, growth, egg production, egg weight, eggshell thickness, fertility, hatchability, or progeny performance. Maximum concentrations in tissues after 8 weeks, in mg/kg FW, in the 500 mg/kg diet group, were 53 in fat, 10 in egg, 9 in liver, and 0.9 in muscle; for the 100 mg/kg group, these values were 21 in fat, 10 in liver, 3 in egg, and 0.5 in muscle; for the 50 mg/kg group, residues were 1.5 in fat, 1 in egg, 0.8 in liver, and 0.2 in muscle. Five weeks after withdrawal from all diets, diflubenzuron was <0.05 mg/kg FW in all tissues	9
Male broiler and layer breed chickens fed 205 mg/kg ration for 98 days beginning at age 1 day	No significant effect on body weight, food consumption, or weight of testes, liver, comb, and feet	10
Male and female layer-breed chickens were fed diets containing up to 250 mg/kg for 58 weeks, including a 26-week laying cycle. Progeny were reared to age 2 weeks.	No significant effect of any dose level on survival, egg production, egg weight, eggshell weight, fertility, hatchability or hatch weight and body weight of progeny. No gross abnormalities in progeny; growth and feathering as in controls	11

[a] 1, Hudson et al. 1984; 2, Farlow 1976; 3, Martinat et al. 1987; 4, Richmond et al. 1979; 5, Opdycke and Menzer 1984; 6, Opdycke et al. 1982b; 7, Gartrell 1981; 8, Miller et al. 1975; 9, Cecil et al. 1981; 10, Kubena 1981; 11, Kubena 1982.

Differences in ability to metabolize diflubenzuron between different strains of domestic chickens may be due to differences in lipid metabolism associated with egg production (Opdycke and Menzer 1984). No comparable data base exists for avian wildlife, and one should be developed through research.

Mammals

No data are available on effects of diflubenzuron on mammalian wildlife. However, results of studies on small laboratory animals and domestic livestock are available (Table 7), and these indicate several trends. Adverse effects levels occurred in dogs fed diets containing 160 mg/kg (6.2 mg/kg BW daily) for 13 weeks (abnormal blood chemistry), in mice given 125 mg/kg BW daily for 30 days (hepatocellular changes), in rabbits fed diets containing 640 mg/kg for 3 weeks (abnormal hemoglobin), and in rats given 5,000 mg/kg BW daily for 13 weeks (abnormal hemoglobin). Accumulations of diflubenzuron occurred in several species. Elevated tissue residues—but no other measurable effects—occurred in cows given 0.05–0.5 mg/kg ration for 28 days or 1–16 mg/kg BW for 4 months, in pigs given a single oral dose of 5 mg/kg BW, and in sheep given a single oral dose of 10 mg/kg BW (Table 7). No observable adverse effect levels occurred in cows given 0.25 mg/kg BW daily for 4 months, in rabbits given 4 mg/kg BW daily on days 6 to 18 of gestation, in dogs fed diets containing 40 mg/kg for 13 weeks (equivalent to 1.6 mg/kg BW daily), in rats fed diets containing 160 mg/kg for 2 years, and in rabbits and rodents given single oral or dermal doses <2,000 mg/kg BW (Table 7).

All available data indicate that diflubenzuron is not a mutagen, teratogen, or carcinogen. Diflubenzuron is not mutagenic, as judged by the results of

1. The mouse lymphoma forward mutation test at the thymidine kinase locus (detects mutations to a nonfunctional thymidine kinase in a line of culture mouse lymphoma cells),
2. The Ames *Salmonella typhimurium* microsome reverse mutation test (ability to produce point gene mutations of a base pair),
3. The mouse micronucleus test (which detects chromosome breakage or chromosome loss from mitotic abnormalities in bone marrow erythrocytes; Mac Gregor et al. 1979), and
4. A DNA damage study with yeast, *Saccharomyces cerevisiae* (Gartrell 1981).

No teratogenicity or reproductive effects were associated with elevated doses of diflubenzuron in all species of mammals tested (Gartrell 1981). Diflubenzuron suppresses melanogenesis and uptake of nucleosides in mouse melanoma cells (Jenkins et al. 1986), and it inhibits growth of experimental tumors in mice, either alone or in combination with $CoCl_2$ (Table 7). Mixed function oxidase, induced by 3-methylcholanthrene, enhances the antitumor properties of diflubenzuron, suggesting that aromatic hydroxylation may be required for tumor growth regulation (Jenkins et al. 1986). The most likely diflubenzuron metabolite that affects tumor growth regulation is the form oxidized at the 2 carbon of the phenyl ring; other metabolites tested (i.e., 4-chlorophenylurea, 3-OH-diflubenzuron) are only marginally effective (Jenkins et al. 1986). Diflubenzuron did not produce tumors in fetal cells of hamsters (*Cricetus* spp.) at whole body doses of 500 mg/kg, and this also suggests a relatively low oncogenic potential (Quarles et al. 1980). Diflubenzuron is not cytotoxic and does not inhibit the synthesis of complex carbohydrates in animal cells, as judged by results of studies with cultured rat glial cells, wherein diflubenzuron was not metabolized to any measurable extent, and more than 98% could be recovered from particulate fractions of whole cells (Bishai and Stoolmiller 1979).

Intestinal absorption of diflubenzuron in laboratory rats, measured as the sum of urinary and biliary excretion, decreases with increasing dose: from 50% at a single oral dose of 4 mg/kg BW to 4% at 900 mg/kg BW. Excretion is almost complete after 75 h; at that time up to 4% of the administered dose is recovered from skinned carcasses (Willems et al. 1980). About 80% of diflubenzuron metabolites excreted by rats seem to have the basic diflubenzuron structure intact. Three metabolites are largely excreted as conjugates in the bile. One metabolite, 2,6-difluorobenzoic acid, is excreted largely in urine. Its counterpart, 4-chlorophenylurea, was not present in urine or bile in appreciable quantity, nor was 4-chloroaniline detected (Willems et al. 1980). Lifetime feeding studies of 4-chloroaniline, a relatively common diflubenzuron metabolite, showed no compound-related effects in laboratory mice and rats (Gartrell 1981).

Oral treatment of sheep and cattle (*Bos* spp.) with diflubenzuron is followed by absorption of the compound through the gastrointestinal tract, metabolism, and elimination of residues through the urine, feces, and, to a limited extent, milk. Intact diflubenzuron is eliminated in the feces of orally dosed cattle and sheep (Ivie 1978). Major metabolites of diflubenzuron excreted by cattle and sheep result from hydroxylation on the difluorobenzoyl and chlorophenyl rings, and by cleavage between the carbonyl and amide groups to produce metabolites that are excreted free or as conjugates (Ivie 1978). Cattle dosed repeatedly with diflubenzuron had detectable residues only in liver and milk. The parent compound, 4-chlorophenylurea, 2,6-difluorobenzoic acid, and 4-chloroaniline compose only 15% of the total residue in liver; the bulk of the residue is not extractable (Gartrell

Table 7. *Diflubenzuron effects on selected mammals.*

Species, mode of administration, dose, and other variables	Effect	Reference[a]
Cattle, *Bos* sp.		
Dermal		
0.125 mg/cm^2 hide, single application, 1% solution to 400 cm^2 skin surface	No absorption through skin; rapid disappearance. Maximum residues in hair, in mg/kg fresh weight (FW), were 128 after 1 week, 19 after 2 weeks, and 4 after 4 weeks. For skin, these values were 0.4, 0.1, and <0.1	1
Diet		
0.05 mg/kg ration for 28 days	No detectable residues in milk and tissues, except liver (0.01 mg/kg FW); liver residues remained detectable after a 7-day withdrawal period	2
0.5 mg/kg ration for 28 days	No detectable residues in milk and tissues, except liver (0.08 mg/kg FW); liver residues remained detectable after a 7-day withdrawal period	2
5 mg/kg ration for 28 days	Liver residue of 0.54 mg/kg FW remained elevated after a 7-day withdrawal period; residues in milk reached 0.013 mg/L within the first few days of feeding and declined to nondetectable (ND) levels after a 4-day withdrawal period	2
Fed diets equivalent to 0.25 mg/kg body weight (BW) daily for 4 months, single animal	No detectable residues in any tissue. Tb 1/2 of 4–5 days in manure; manure gave >95% control of larvae of the face fly, *Musca autumnalis*	3
Fed diet equivalent to 1 mg/kg BW daily for 4 months, single animal	No detectable residues in any tissue except omental fat (0.1 mg/kg FW). No houseflies (*Musca domestica*) or face flies developed in manure	3
Fed diet that increased from 1 mg/kg BW daily to 8 mg/kg BW over a 2-month period, then 16 mg/kg BW daily for 3 months	No detectable diflubenzuron residues in heart, muscle, or kidney; 130 μg/kg FW in liver; about 250 μg/kg FW in subcutaneous fat	3
Holstein bull calves fed diet equivalent to 2.8 mg/kg BW daily for first 7 months, then 1 mg/kg BW daily for 6–12 months	No effect on weight gain, serum testosterone at age 11 months, libido, sperm mobility, semen volume, or sperm concentration. No histopathology of liver, lung, kidney, or spleen. No tissue residues—except for one bull slaughtered at age 5 months: <20 μg/kg FW in muscle, 20 in liver and kidney, 40 in subcutaneous fat, and 80 in renal and omental fat	4
Fed diet equivalent to 8 mg/kg BW daily for 4–5 months, single animal	No detectable residues in milk	3
Fed diet equivalent to 16 mg/kg BW daily for 4–5 months, single animal	Maximum concentrations recorded were 20 μg/L in milk and 250 μg/kg FW in body fat. No obvious adverse effects on feeding behavior	3

Table 7. *Continued.*

Species, mode of administration, dose, and other variables	Effect	Reference[a]
Oral		
10 mg/kg BW, single-dose to a lactating cow	Extensively metabolized in 4 days; almost all totally excreted in 7 days: about 85% in feces, 15% in urine, 0.2% in milk. At 7 days, liver contained 2.9 mg/kg FW, skin 0.4, and all other tissues <0.4	1
Dog, *Canis familiaris*		
Fed diets containing 10, 20, 40, or 160 mg/kg (equivalent to to 0.42, 0.84, 1.64, or 6.24 mg/kg BW daily) for 13 weeks	Abnormal hemoglobin levels in 160-mg/kg group after 6 weeks; no other abnormal findings or histopathology observed in any group at 13 weeks	2
Angora goat, *Capra* sp.		
30 mL of 2% diflubenzuron solution applied dermally 6 weeks after shearing, 25-kg females	Protected against Angora goat biting lice (*Bovicola limbatus*) for up to 18 weeks	5
Domestic mouse *Mus* sp.		
Diet		
4, 8, 16, or 50 mg/kg ration for 80 weeks	Increase in tumors in females at the 16-mg/kg level	2
Intraperitoneal injection		
3 daily injections of 1.2 mg, equivalent to 144 mg/kg BW, tumor-bearing strain	Tumors conditioned with $CoCl_2$ then treated with diflubenzuron showed a 75% reduction in rate of tumor increase	6
5 daily injections of 20 mg (total of 100 mg, equivalent to 4,000 mg/kg BW), C57BL/6 strain with B16 melanomas	Initial antitumor activity, as judged by 11–20% decrease in tumor volume, and a 2–3 day increase in tumor doubling time. But at midtreatment, tumors regained control rate of volume increase	6
2,150 mg/kg BW	Insufficient to kill 50%	7
Oral		
Adult males given 125, 500, or 2,000 mg/kg BW daily for 30 days	Hepatocellular changes at all dose levels, including histopathology and altered activities of glutathione S-transferase enzymes	8
>4,640 mg/kg BW	Acute oral LD50	2,7,8
Rabbit, *Oryctolagus* sp.		
Dermal		
2,000 mg/kg BW	Insufficient to kill 50%	7
Diet		
Males given 640 mg/kg feed for 18–21 days	Abnormal hemoglobin	2
In vitro studies		

Table 7. *Continued.*

Species, mode of administration, dose, and other variables	Effect	Reference[a]
Up to 5 mg/L	Protein and RNA synthesis rates were significantly stimulated in liver, and inhibited in muscle in a dose-dependent manner. Maximum effect in both tissues occurred at 5 mg/L for protein synthesis and 0.2 mg/L for RNA synthesis	9
Oral		
Females given 1, 2, or 4 mg/kg BW daily on days 6–18 of gestation	No compound-related maternal toxicity or birth defects	2
Sheep, *Ovis aries*		
Dermal		
Merrino sheep exposed to mass-released gravid females of the sheep blowfly (*Lucilia cuprina*)—a severe ectoparasite in Australia that may kill—in a fly-proof animal house after dermal application of 1,000, 1,500, or 2,500 mg diflubenzuron/L; sheep thoroughly wetted twice during 4 days	1,000 mg/L protected against fly strike for at least 110 days; 1,500 mg/L protected until end of trial at 170 days; 2,500 mg/L provided excellent protection against severe infestation. No resistance to diflubenzuron was acquired by blowflies	10
Oral		
Single dose of 10 mg/kg BW	Residues after 7 days, in mg/kg FW, were about 3 in liver, 0.4 in kidney, and <0.2 in all other tissues	1
Single dose of 500 mg/kg BW	In 4 days, bile accounted for 36% of diflubenzuron metabolites excreted, feces 32%, and urine 24%; in 7 days, feces were the major pathway	1
Laboratory white rat, *Rattus* sp.		
Diet		
Fed 10, 20, 40, or 160 mg/kg ration for three generations	No effect on fetotoxicity or teratogenicity	2
Fed 10, 20, 40, or 160 mg/kg ration for 2 years	No compound-related effects	2
Oral		
Females given 1, 2, or 4 mg/kg BW daily on days 6–15 of gestation	No compound-related maternal toxicity or birth defects	2
4 mg/kg BW, single dose	Intestinal absorption of 50%	11

Table 7. *Continued.*

Species, mode of administration, dose, and other variables	Effect	Reference[a]
5 mg/kg BW, single dose	72-93% excreted in 6 days, mostly in feces	11
900 mg/kg BW, single dose	Intestinal absorption of 4%	11
>4,640 mg/kg BW	Acute oral LD50	2,7
Males given 5,000 mg/kg BW daily for 13 weeks	Abnormal hemoglobin on days 1-4, and on day 8	2
Swine, *Sus* sp.		
Adult female pig given single oral dose of 5 mg/kg BW and observed for 11 days	By 11 days, 82% of dose was excreted unchanged in feces, and 5% in urine as metabolites (4-chlorophenylurea, 2, 6-difluorobenzoic acid, 4-chloroaniline, and 2,6-difluorobenzamide). Tissue residues, in mg/kg FW, ranged from ND in bone to 0.04-0.09 in stomach wall, brain, pancreas, small intestine, blood, heart, muscle, and ovary; from 0.11-0.2 for large intestine, subcutaneous fat, lymph, lung, and kidney; and from 0.23 to 0.4 in liver, omental fat, and gall bladder	12

[a] 1, Ivie 1978; 2, Gartrell 1981; 3, Miller et al. 1976; 4, Miller et al. 1979; 5, Miller et al. 1985; 6, Jenkins et al. 1986; 7, Poplyk 1989; 8, Young et al. 1986; 9, El-Sebae et al. 1988; 10, Hughes and Levot 1987; 11, Willems et al. 1980; 12, Opdycke et al. 1982a.

1981). Dietary levels of 5 mg/kg ration produce low (13 μg/L), but detectable, diflubenzuron concentrations in milk of cattle (Gartrell 1981).

The major hydroxylated diflubenzuron metabolite in cow milk (N-[[(4-chlorophenyl) amino] carbonyl] -2,6-difluoro-3-hydroxybenzamide) when fed to white rats is rapidly excreted with little biotransformation (Ivie 1978).

Metabolism of diflubenzuron by mammals and birds probably occurs by way of hydroxylation, conjugation, and cleavage of the urea moiety (Opdycke et al. 1982a); however, interspecies differences are considerable. In cows, for example, the major identified metabolic transformation is hydroxylation at the 3 position of the 2,6-difluorobenzoyl ring. In sheep, however, major metabolites arise through cleavage of the amide bond at the benzoyl carbon to produce 2,6-difluorobenzoic acid, which is excreted in the urine either free or conjugated with glycine (Ivie 1978). The major diflubenzuron metabolite in cow urine is 2,6-difluoro-3-hydroxydiflubenzuron, accounting for 45%, and in feces 18%; unchanged diflubenzuron accounts for 43% of the administered dose in cow feces. In sheep urine, 2,6-difluorobenzoic acid and 2,6-difluorohippuric acid account for 57%; in sheep feces, unchanged diflubenzuron is 97% (Ivie 1978). In swine, the majority of the administered dose is eliminated in feces unchanged; the urine contains mostly metabolites, indicating that most of the absorbed diflubenzuron is metabolized (Opdycke et al. 1982a).

Recommendations

Since diflubenzuron toxicity seems to be similar in both insects and crustaceans, extreme care must be taken when this compound and other chitin synthesis inhibitors are used for insect control in areas where aquatic crustaceans occur. Otherwise, ecological instability may result, with consequences for feeding, metabolism, growth, reproduction, and survival of numerous nontarget organisms (Christiansen 1986). Specifically, diflubenzuron use in saltmarsh mosquito breeding areas or on agricultural lands less than 5 km from coastal areas is not recommended because of concerns that runoff may reach the adjacent estuaries, which are the primary hatcheries for many economically important species of crustaceans (Costlow 1979; Cunningham 1986; Cunningham and Myers 1986). Also, diflubenzuron concentrations in seawater should not exceed 0.1 μg/L, the minimum concentration known to produce measurable behavioral changes in estuarine crustacean larvae (Cunningham and Myers 1986).

If diflubenzuron and other insect growth regulators continue to be used near productive aquatic habitats, then food chain transfer studies are recommended. High accumulations of diflubenzuron by aquatic algae—up to 4.5 mg/kg DW in some cases (Booth and Ferrell 1977)—strongly implicate food chain transfer as a potential mechanism of contaminant transfer in aquatic invertebrate food webs. To protect certain fishes, diflubenzuron use to control copepod vectors of human disease—including various species of *Cyclops*—is not recommended in areas where these fishes breed or feed on *Cyclops* (Rao and Paul 1988).

For control of cotton pests, including the boll weevil, a maximum recommended treatment schedule is 421 g diflubenzuron per ha, applied six times, usually weekly, during the growing season (Bull 1980). Honey bees (*Apis mellifera*) in heavily sprayed areas, however, may experience adverse effects if their diets exceed 1 mg diflubenzuron per kg FW (Stoner and Wilson 1982). Diflubenzuron inhibits house fly development in poultry manure. A recommended cost-effective fly control program in poultry houses involves the feed-through method (5 mg diflubenzuron per kg FW poultry diet) during hot, wet summers for 3–4 months, coupled with good sanitation and good manure management (Giga 1987).

For protection of domestic cattle, feeds should contain <0.05 mg diflubenzuron per kg FW; cottonseed may be added to cattle diets provided that diflubenzuron concentrations in the seed do not exceed 0.2 mg/kg FW and that cottonseed composes <17% of the total diet bulk (Gartrell 1981).

Diflubenzuron causes biochemical upset, as judged by lowered testosterone levels in chickens and rats (EPA 1979), altered glutathione S-transferase activity in mouse liver (which adversely affects the ability to detoxify foreign substances by way of conjugation; Young et al. 1986), and disrupted hydroxylamine activity in human infants (EPA 1979). Additional research seems needed on biochemical alterations induced by diflubenzuron.

No diflubenzuron criteria are currently recommended for protection of avian and mammalian wildlife. All data available suggest that wildlife species are about as tolerant to diflubenzuron as are domestic poultry and livestock; however, the wildlife data base seems inadequate for practicable criteria formulation.

Anti-cancer properties of diflubenzuron require elucidation. The indication that one or more hydroxylated forms of diflubenzuron can regulate growth of mouse tumor cells provides a basis for further studies to identify and isolate the most active analog of this compound, and it suggests that other benzoylphenyl ureas may have similar properties (Jenkins et al. 1986).

Diflubenzuron has a Surveillance Index Classification of Class IV, indicating a sufficiently low hazard potential to human health from toxicological and exposure standpoints to justify only minimal monitoring efforts (Gartrell 1981). Human cancer risk of lifetime dietary exposure to diflubenzuron in a worst case scenario is considered slight (EPA 1979). Diflubenzuron has little potential for human dietary exposure because of its limited use on cotton and the low residues measured on cottonseed, meat, milk, poultry, and eggs (Gartrell 1981). For protection of human health, tolerances of <0.05 mg/kg FW have been set for fat, meat, meat byproducts, poultry, milk, dairy products, and eggs, and <0.2 mg/kg FW for cottonseed (EPA 1979). These foods compose about 45% of the average human diet. If all of these foods bore residues at the tolerance level, they would contribute 0.035 mg daily on the basis of 1.5 kg food eaten daily. For a 60-kg adult, the theoretical maximum residue concentration would be 0.6 μg/kg BW daily. Tolerances would be approached only when maximum quantities of cottonseed fraction (i.e., hulls, meal, soapstock), all bearing tolerance-level residues, are incorporated into livestock diets. At present, however, no acceptable daily intake level in humans has been established (Gartrell 1981).

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